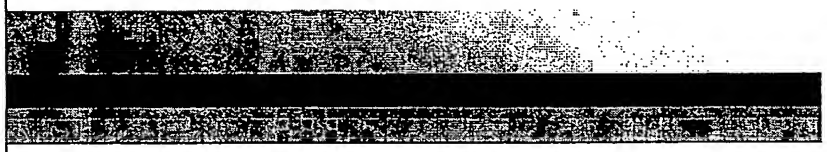
GeneChip®
Expression Analysis Technical Manual

For Cartridge Arrays Using the GeneChip® Array Station

P/N 702064 Rev. 1

For research use only.
Not for use in diagnostic procedures.

Contents



Chapter 1 Overview

Chapter 1

Introduction

Welcome to the Affymetrix® GeneChip® Array Station. This system uses robotic technology to automate many of the labor intensive tasks used in eukaryotic target preparation.

This chapter describes the assay procedures recommended for eukaryotic target labeling for expression analysis using GeneChip® brand probe arrays. By following the protocols and using high-quality starting materials, a sufficient amount of biotin-labeled complementary RNA (cRNA) target can be obtained for hybridization. The reagents and protocols have been developed and optimized specifically for use with the GeneChip Array Station.

The One-Cycle Eukaryotic Target Labeling Assay experimental outline is represented in Figure 1.1. Total RNA (1 µg to 2 µg) is first reverse transcribed using a T7-Oligo(dT) Promoter Primer in the first-strand cDNA synthesis reaction. Following RNase H-mediated second-strand cDNA synthesis, the double-stranded cDNA is purified and serves as a template in the subsequent *in vitro* transcription (IVT) reaction. The IVT reaction is carried out in the presence of T7 RNA Polymerase and a biotinylated nucleotide analog/ribonucleotide mix for cRNA amplification and biotin labeling. The biotinylated cRNA targets are then cleaned up, fragmented, and hybridized to GeneChip expression arrays.

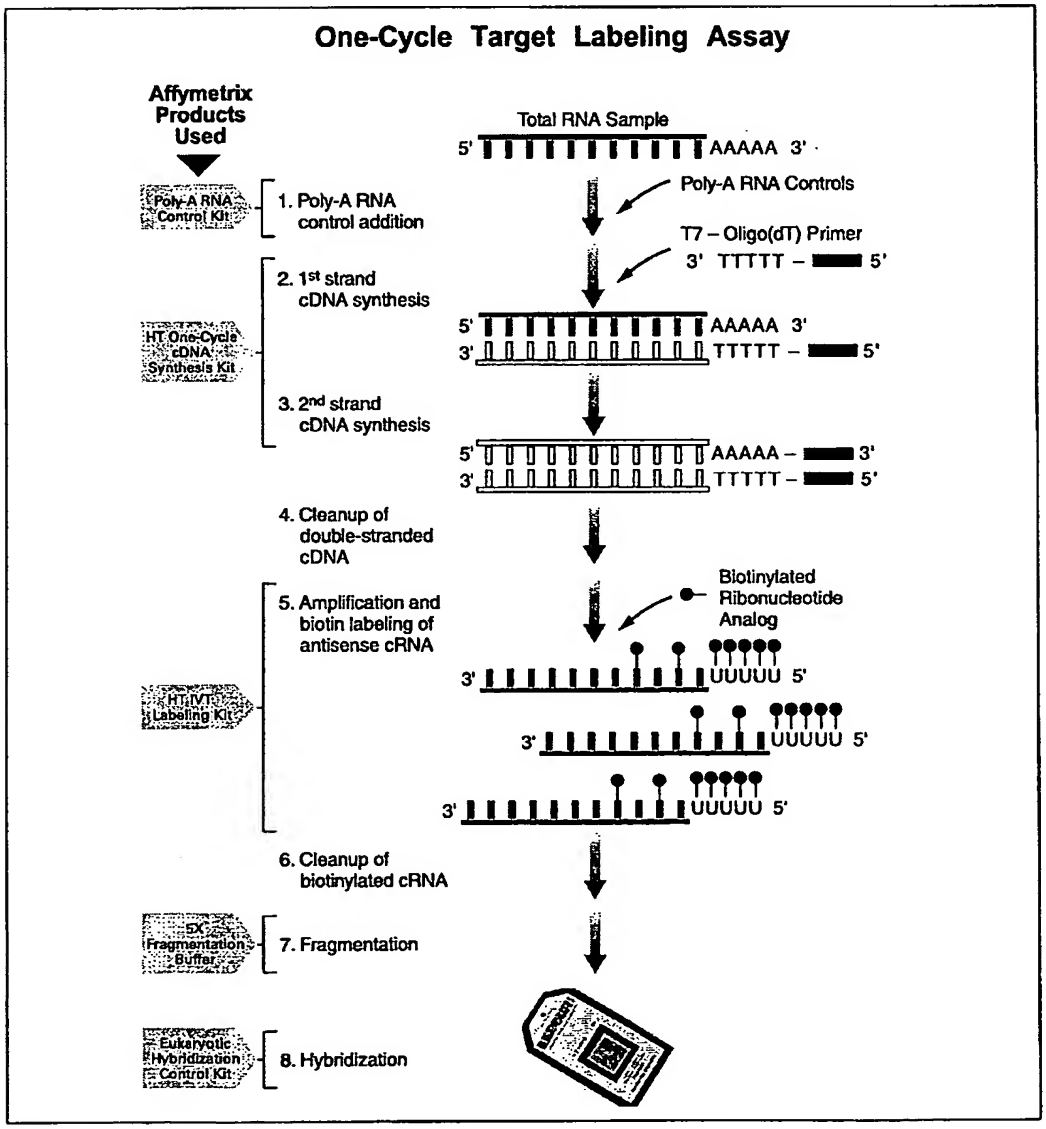


Figure 1.1
GeneChip® Eukaryotic Labeling Assays for Expression Analysis Using GeneChip® Array Station

SUMMARY OF THE ARRAY STATION RUN

Target preparation on the Array Station takes approximately 22 hours and requires two human interventions: sample preparation and cRNA quantitation. At the end of target preparation, the sample is ready for hybridization onto a cartridge. Hybridization takes 16 hours and additional time is required for washing, staining, and scanning. This additional time is dependent on the number of samples processed, instrument capacity, and array type. The typical workflow and chapter references are provided below.

1. Isolate high quality total RNA (refer to Chapter 2).
2. Prepare reagents required for target preparation (refer to Chapter 3).
3. Perform Array Station system check (refer to Chapter 4).
4. Set-up first deck layout required for cDNA synthesis reaction through purification of cRNA (refer to Chapter 4).
5. Set-up second deck layout required for the quantitation and normalization steps and prepare the hybridization- ready sample (refer to Chapter 4).
6. Denature sample and hybridize overnight onto GeneChip® cartridge arrays (refer to Chapter 6).
7. Wash and stain cartridge arrays using the Fluidics Station 450 (refer to Chapter 6).
8. Scan cartridge arrays using the GeneChip® Scanner 3000 (refer to Chapter 6).

Array Station Subsystems

The array station is composed of both mechanical and software subsystems. Refer to the *GeneChip® Array Station User's Manual* (P/N 701859) for more information.

TYPICAL CORE SYSTEM

Figure 1.2 illustrates the core mechanical subsystems of the Array Station including the Caliper Sciclone workstation, Bio-Rad DNA Engine® Thermal Cycler, and Twister® II microplate handler. For detailed information on the various mechanical components of the system, please refer to the *GeneChip® Array Station User's Manual* (P/N 701859).

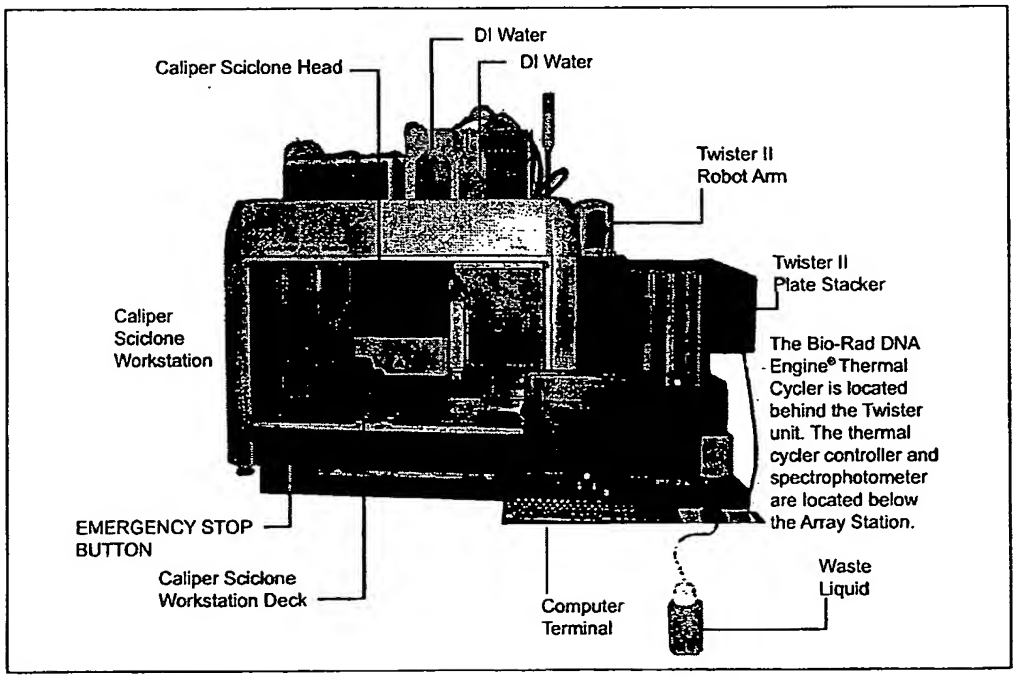


Figure 1.2
The GeneChip® Array Station

The iLink™ control software provides a graphical user interface to run and track the various mechanical components. The iLink software acts as the top-level application that communicates via the Instrument Control Program (ICP) to schedule and control these instruments. See Figure 1.3 for a schematic of the software product configuration for the Array Station system.

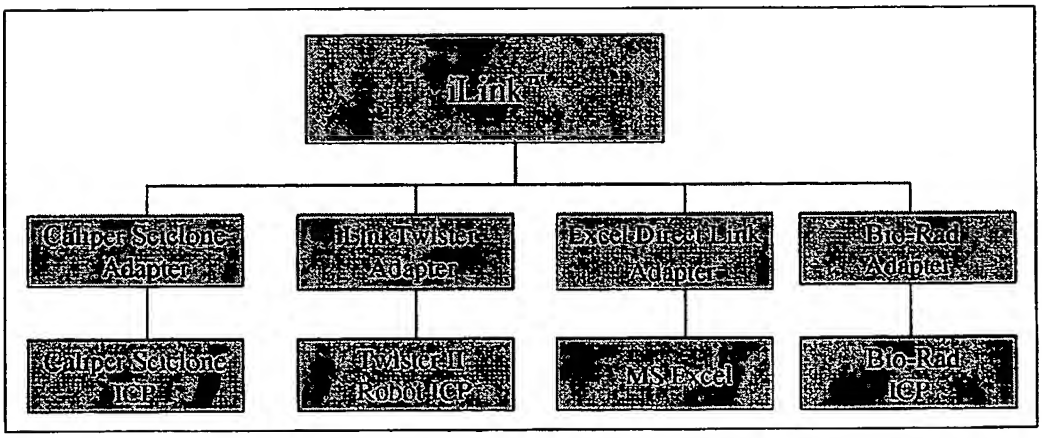


Figure 1.3
Array Station Product Software Configuration

User Documentation and Quick Reference Cards

The operation of the Array Station robotic system requires familiarity with the following user documentation.

Those manuals that are relevant will depend on your system configuration. For the Array Station configuration, the following list presents the relevant guides:

1. *GeneChip® Array Station Site Prep Guide* (P/N 707020)
2. *GeneChip® Array Station User's Manual* (P/N 701859)
3. *iLink™ System User's Manual*, section 1: "Operator Instructions" (from Caliper Life Sciences).
4. *GeneChip® Array Station Deck Layout Quick Reference Card* (P/N 707013)
5. *GeneChip® Array Station Reagent Preparation Quick Reference Card* (P/N 707032)
6. *Affymetrix GeneChip® Operating Software User's Guide* (P/N 701439): if you are using Affymetrix GeneChip analysis software, you should be familiar with Affymetrix' GeneChip Operating System in order to interpret the assay results.

The following manuals are for operator reference only. You should never tamper or alter the initial Affymetrix protocols or scripts.

1. *iLink™ System User's Manual*, section 2: "Integrator Instructions" (from Caliper Life Sciences).
2. *Caliper Sciclone User's Manual* (from Caliper Life Sciences).
3. *Bio-Rad MJ Thermal Cycler ICP Guide* (from Bio-Rad/MJ Research).
4. *Excel DirectLink* (from Caliper Life Sciences).

Regulatory Compliance

We, Affymetrix

4G Crosby Drive

Bedford, MA 01730

USA

declare under sole responsibility that the Affymetrix GeneChip® Array Station and associated Workstation with software, is Manufactured in the United States of America, with U.S. and Non-U.S. components.

This device complies with Part 15 of FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) This device must accept any interference received, including interference that may cause undesired operation.

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulation.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel broullier du Canada.

Regulatory Agency	Certification
	92AA
	
	
	Compliant with directive 2002/96/EC (WEEE)

Chapter 2

RNA Preparation

Chapter 2

Total RNA Isolation for One-Cycle Eukaryotic Target Labeling Assay

This chapter describes the general requirements for RNA isolation methods and poly-A control preparation for spiking into your RNA sample.

IMPORTANT ! **The quality of the RNA is essential to the overall success of the analysis. Since the most appropriate protocol for the isolation of RNA can be source dependent, we recommend using a protocol that has been established for the tissues or cells being used. In the absence of an established protocol, using one of the commercially available kits designed for RNA isolation is suggested.**

When using a commercial kit, follow the manufacturer's instructions for RNA isolation.

MATERIALS LIST

Total RNA Isolation Reagents

- TRIzol® Reagent: Invitrogen Life Technologies, P/N 15596-018, or QIAzol™ Lysis Reagent: QIAGEN, P/N 79306
- RNeasy® Mini Kit: QIAGEN, P/N 74104

Miscellaneous Reagents

- 80% ethanol (stored at –20°C)
- Pellet Paint®: Novagen, P/N 69049-3 (optional)
- Glycogen: Ambion, P/N 9510 (optional)
- 3 M Sodium Acetate (NaOAc): Sigma-Aldrich, P/N S7899

ISOLATION OF RNA FROM YEAST

Total RNA

Quality total RNA has been isolated successfully from yeast cells using a hot phenol protocol described by Schmitt, *et al. Nucl Acids Res* 18:3091-3092 (1990).

ISOLATION OF RNA FROM ARABIDOPSIS

Total RNA

TRIzol® Reagent from Invitrogen Life Technologies has been used to isolate total RNA from Arabidopsis. Follow the instructions provided by the supplier and, when necessary, use the steps outlined specifically for samples with high starch and/or high lipid content. QIAzol™ Lysis Reagent from QIAGEN can also be used.

ISOLATION OF RNA FROM MAMMALIAN CELLS OR TISSUES

Total RNA

High-quality total RNA has been successfully isolated from mammalian cells (such as cultured cells and lymphocytes) using the RNeasy® Mini Kit from QIAGEN.

If mammalian tissue is used as the source of RNA, it is recommended to isolate total RNA with a commercial reagent, such as TRIzol® or QIAzol™ reagent.

IMPORTANT !

If going directly from TRIzol- or QIAzol™-isolated total RNA to cDNA synthesis, it may be beneficial to perform a second cleanup on the total RNA before starting. After the ethanol precipitation step in the Lysis Reagent extraction procedure, perform a cleanup using the QIAGEN RNeasy Mini Kit. Much better yields of labeled cRNA are obtained from the *in vitro* transcription-labeling reaction when this second cleanup is performed.

PRECIPITATION OF RNA

Total RNA

It is not necessary to precipitate total RNA following isolation or cleanup with the RNeasy Mini Kit. Adjust elution volumes from the RNeasy column to prepare for cDNA synthesis based upon expected RNA yields from your experiment. Ethanol precipitation is required

following TRIzol or QIAzol reagent isolation and hot phenol extraction methods; see methods on page 15 for details.

PRECIPITATION PROCEDURE

1. Add 1/10 volume 3 M NaOAc, pH 5.2, and 2.5 volumes ethanol.*
2. Mix and incubate at -20°C for at least 1 hour.
3. Centrifuge at ≥ 12,000 x g in a microcentrifuge for 20 minutes at 4°C.
4. Wash pellet twice with 80% ethanol.
5. Air dry pellet. Check for dryness before proceeding.
6. Resuspend pellet in DEPC-treated H_2O. The appropriate volume for resuspension depends on the expected yield and the amount of RNA required for the cDNA synthesis. Please read ahead to the cDNA synthesis protocol in order to determine the appropriate resuspension volume at this step.

*Addition of Carrier to Ethanol Precipitations

Adding carrier material has been shown to improve the RNA yield of precipitation reactions.

- Pellet Paint®
  Addition of 0.5 µL of Pellet Paint per tube to nucleic acid precipitations makes the nucleic acid pellet easier to visualize and helps reduce the chance of losing the pellet during washing steps. The pellet paint does not appear to affect the outcome of subsequent steps in this protocol; however, it can contribute to the absorbance at 260 nm when quantifying the total RNA.
- Glycogen
  Addition of 0.5 to 1 µL of glycogen (5 mg/mL) to nucleic acid precipitations aids in visualization of the pellet and may increase recovery. The glycogen does not appear to affect the outcome of subsequent steps in this protocol.

QUANTITATION OF RNA

Quantify RNA yield by spectrophotometric analysis using the convention that 1 absorbance unit at 260 nm equals 40 µg/mL RNA.

- The absorbance should be checked at 260 and 280 nm for determination of sample concentration and purity.
- The A_{260}/A_{280} ratio should be close to 2.0 for pure RNA (ratios between 1.9 and 2.1 are acceptable).
- Integrity of total RNA samples can also be assessed qualitatively on an Agilent 2100 Bioanalyzer. Refer to Figure 2.1 for an example of good-quality total RNA sample.

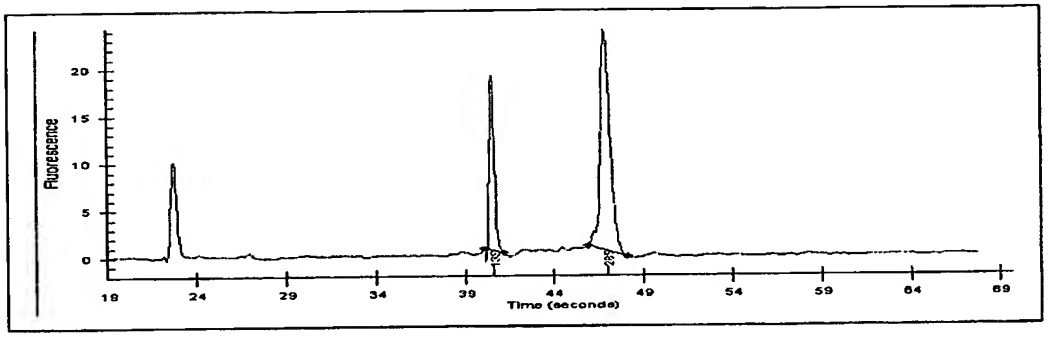


Figure 2.1
Electropherogram (from the Agilent 2100 Bioanalyzer) for HeLa Total RNA. For a high-quality total RNA sample, two well-defined peaks corresponding to the 18S and 28S ribosomal RNAs should be observed, similar to a denaturing agarose gel, with ratios approaching 2:1 for the 28S to 18S bands.

PURIFIED TOTAL RNA

If you have less than 20 µL of purified total RNA, pipet 5 µL of the RNA sample (containing 1 to 2 µg) to a Bio-Rad 96-Well Hard-Shell PCR Plate and select **Manual Sample Transfer** in the iLink software. For more information, please see the software protocol on page 63.

If you have 20 µL of purified total RNA in a 96 well Greiner U-bottom plate, you may use the Array System to transfer the 5 µL total RNA to the Bio-Rad 96-Well Hard-Shell PCR Plate. Please refer to Appendix B for information on the Automated Sample Transfer.

PREPARATION OF POLY-A RNA CONTROLS FOR ONE-CYCLE cDNA SYNTHESIS (SPIKE-IN CONTROLS)

Reagents and Equipment

- GeneChip® Eukaryotic Poly-A RNA Control Kit: Affymetrix, P/N 900433

Designed specifically to provide exogenous positive controls to monitor the entire eukaryotic target labeling process, a set of poly-A RNA controls is supplied in the GeneChip Eukaryotic Poly-A RNA Control Kit.

Each eukaryotic GeneChip probe array contains probe sets for several *B. subtilis* genes that are absent in eukaryotic samples (*lys, phe, thr*, and *dap*). These poly-A RNA controls are *in vitro* synthesized, and the polyadenylated transcripts for the *B. subtilis* genes are pre-mixed at staggered concentrations. The concentrated Poly-A Control Stock can be diluted with the Poly-A Control Dil Buffer and spiked directly into RNA samples to achieve the final dilutions (relative to estimated copy number of total mRNA population) summarized in Table 2.1.

Table 2.1
Final Dilutions of Poly-A RNA Controls in Samples

Poly-A RNA Spike	Final Dilution (estimated ratio of copy number)
lys	1:100,000
phe	1:50,000
thr	1:25,000
dap	1:6,667

The controls are then amplified and labeled together with the samples. Examining the hybridization intensities of these controls on GeneChip arrays helps to monitor the labeling process independently from the quality of the starting RNA samples. Anticipated relative signal strength follows the order of *lys* < *phe* < *thr* < *dap*.

The Poly-A RNA Control Stock and Poly-A Control Dil Buffer are provided with the kit to prepare the appropriate serial dilutions based on Table 2.2. This is a guideline when 1 or 2 µg of total RNA is used as starting material.

IMPORTANT !

Use non-stick RNase-free microfuge tubes to prepare all of the dilutions.

Table 2.2
Serial Dilutions of Poly-A RNA Control Stock

Starting Amount		Serial Dilutions			Spike-in Volume
Total RNA	mRNA	First	Second	Third	
1 µg		1:20	1:50	1:50	2 µL
2 µg		1:20	1:50	1:25	2 µL

TIP

Avoid pipetting solutions less than 2 µL in volume to maintain precision and consistency when preparing the dilutions.

For example, to prepare the poly-A RNA dilutions for 1 µg of total RNA:

1. Add 2 µL of the Poly-A Control Stock to 38 µL of Poly-A Control Dil Buffer for the First Dilution (1:20).
2. Mix thoroughly and spin down to collect the liquid at the bottom of the tube.
3. Add 2 µL of the First Dilution to 98 µL of Poly-A Control Dil Buffer to prepare the Second Dilution (1:50).
4. Mix thoroughly and spin down to collect the liquid at the bottom of the tube.
5. Add 2 µL of the Second Dilution to 98 µL of Poly-A Control Dil Buffer to prepare the Third Dilution (1:50).

6. Mix thoroughly and spin down to collect the liquid at the bottom of the tube.
7. Add 2 µL of this Third Dilution to 1 µg of sample to total RNA directly. The final volume of the total RNA with the diluted poly-A controls should not exceed 5 µL.

NOTE **The First Dilution of the poly-A RNA controls can be stored up to six weeks in a non- frost-free freezer at –20°C and frozen-thawed up to eight times.**

Chapter 3

Reagent Preparation

Chapter 3

Introduction

This chapter describes the reagent preparation for the one-cycle eukaryotic target labeling assay. This involves manually preparing and loading strip tubes with reagent mixtures as well as filling the water and ethanol reservoirs. The initial reagent setup must be preformed prior to starting target preparation. Figure 3.1 illustrates the order of reagents loaded into the cold block.

IMPORTANT !

Before loading the strip tubes, please remove all bubbles from the bottom of the tubes by gently pipetting the solutions up and down.

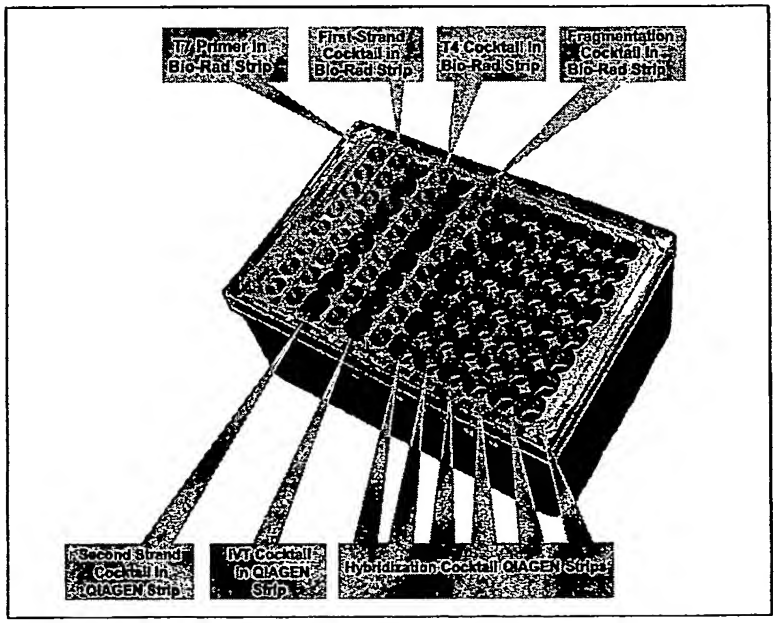


Figure 3.1
Reagent Cold Block Description

In the following sections, the components for 24-, 48-, and 96-sample master mixes are provided. Refer to Appendix A for detailed information on other reaction configurations.

Reagents and Materials Required

The following reagents and materials have been tested and evaluated by Affymetrix. Information and part numbers listed are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix D of this manual.

IMPORTANT **Do not store enzymes in a frost-free freezer.**

REAGENTS AND EQUIPMENT

- GeneChip® HT One-Cycle cDNA Synthesis Kit: Affymetrix, P/N 900687[1]
- GeneChip® HT IVT Labeling Kit; Affymetrix: P/N 900688
- GeneChip® Eukaryotic Hybridization Control Kit: Affymetrix, P/N 900454 (30 reactions) or P/N 900457 (150 reactions), contains Control cRNA and Control Oligo B2
- Control Oligo B2 (3 nM): Affymetrix, P/N 900301 (can be ordered separately)
- Nuclease-free Water: Ambion, P/N 9932
- 5 M NaCl, RNase-free, DNase-free: Ambion, P/N 9760G
- RNase-Free 1.5 mL microfuge tube: Ambion, P/N 12400
- MES Hydrate SigmaUltra: Sigma-Aldrich, P/N M5287
- MES Sodium Salt: Sigma-Aldrich, P/N M5057
- DMSO, Sigma-Aldrich: P/N D5879
- EDTA Disodium Salt, 0.5 M solution (100 mL): Sigma-Aldrich, P/N E7889
- Herring Sperm DNA: Promega Corporation, P/N D1811
- Low-Profile 0.2 mL PCR 8-Tube Strips: Bio-Rad, P/N TLS-0801
- Elution Strip Tubes, 0.85 mL: QIAGEN, P/N 19588

[1] *Users who do not purchase this Kit may be required to obtain a license under U.S. Patent Nos. 5,716,785, 5,891,636, 6,291,170, and 5,545,522 or to purchase another licensed kit.*

- BD Falcon™ Test Tube, 5 mL: VWR International, P/N 60819-728 or a 1.5 mL microcentrifuge vials: USA Scientific, P/N 1415-2600.
- BD Falcon™ Test Tube, 14 mL: VWR International, P/N 60819-761
- Polypropylene Centrifuge Tubes with Caps, 50 mL: VWR International, P/N 20171-028
- Bovine Serum Albumin (BSA) solution (50 mg/mL): Invitrogen Life Technologies, P/N 15561-020
- Surfact-Amps 20 (Tween-20), 10%: Pierce Chemical, P/N 28320

Before You Begin

NOTE Determine the number of reactions to run for target preparation and then prepare the reagents according to the number of reactions indicated in the tables provided in the following steps.

IMPORTANT The tables in this guide are suited for RNA samples loaded in the total RNA plate columnwise.

The volume per strip tube for each reagent is adjusted to sufficiently accommodate the requirements of the GeneChip® Array Station.

Assemble the Peltier adapter and pre-chilled cold block. Ensure that the unit is chilled to 4°C prior to loading the reagent strip tubes containing the prepared reagent master mixes.

Set up the cold block on the GeneChip® Array Station deck before preparing the reagent master mixes.

Reagent master mixes should not be vortexed. Gently pipet the solution to ensure reagents are uniformly mixed.

The amount added to the wells of the strip tubes is slightly less than the total volume of prepared reagent. This is needed to compensate for technical differences that may result from pipette and operator variation, and the properties of the reagents (i.e., viscosity).

Step 1: T7 Primer Master Mix

PROCEDURE

NOTE **Refer to Table 3.1 for the T7 Primer Master Mix composition.**

1. Obtain an RNase-free 1.5 mL microfuge tube and label as "T7 Primer."
2. Obtain a Bio-Rad Low-Profile PCR Tube Strip and label as "T7."
3. Add the components listed in Table 3.1 to the microfuge tube and mix well.
4. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the PCR strip tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tube to column 1 of the cold block.

Table 3.1
T7 Primer Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
T7-Oligo(dT) Primer, 50 µM	1.0 µL	32.0 µL	57.0 µL	114.0 µL
Nuclease-free Water	4.0 µL	127.9 µL	228.0 µL	456.0 µL
Total Volume	5.0 µL	159.9 µL	285.0 µL	570.0 µL
Volume per Strip Tube Well		18.5 µL	34.0 µL	69.3 µL

Step 2: First-Strand cDNA Synthesis Master Mix

PROCEDURE

NOTE **Refer to Table 3.2 for the First-Strand cDNA Synthesis Cocktail composition.**

1. Obtain an RNase-free 1.5 mL microfuge tube and label as "1st Strand."
2. Obtain a Bio-Rad Low-Profile PCR Tube Strip and label as "1st."
3. Add the components listed in Table 3.2 to the microfuge tube and mix well.
4. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the PCR strip tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tube to column 2 of the cold block.

Table 3.2
First-Strand cDNA Synthesis Cocktail for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
5X 1st Strand Reaction Mix	4.0 µL	114.0 µL	228.0 µL	456.0 µL
DTT, 0.1 M	2.0 µL	57.0 µL	114.0 µL	228.0 µL
dNTP Mix, 10 mM	1.0 µL	28.5 µL	57.0 µL	114.0 µL
SuperScript™ II	1.0 µL	28.5 µL	57.0 µL	114.0 µL
Nuclease-free Water	2.0 µL	57.0 µL	114.0 µL	228.0 µL
Total Volume	10.0 µL	285.0 µL	570.0 µL	1,140.0 µL
Volume per Strip Tube Well		33.5 µL	69.3 µL	140.5 µL

Step 3: Second-Strand cDNA Synthesis Master Mix

PROCEDURE

NOTE **Refer to Table 3.3 for the Second-Strand cDNA Synthesis composition.**

1. Obtain a 5 mL BD Falcon Test Tube with a clip-on cap for large volumes or a 1.5 mL microfuge tube for smaller volumes (for 32 reactions or less) and label as "2nd Strand."
2. Obtain an 0.85 mL QIAGEN Elution Strip Tube and label as "2nd."
3. Add the components listed in Table 3.3 to the BD Falcon Test Tube or the microfuge tube and mix well.
4. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the QIAGEN Elution Strip Tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tube to column 3 of the cold block.

Table 3.3
Second-Strand cDNA Synthesis

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
5X 2nd Strand Reaction Mix	30.0 µL	810.0 µL	1,620.0 µL	3,240.0 µL
dNTP Mix, 10 mM	3.0 µL	81.0 µL	162.0 µL	324.0 µL
E. coli DNA Ligase, 10 unit/µL	1.0 µL	27.0 µL	54.0 µL	108.0 µL
E. coli DNA Polymerase I, 10 unit/µL	4.0 µL	108.0 µL	216.0 µL	432.0 µL
RNase H, 2 unit/µL	1.0 µL	27.0 µL	54.0 µL	108.0 µL
Total Volume	39.0 µL	1,053.0 µL	2,106.0 µL	4,212.0 µL
Volume per Strip Tube Well		129.0 µL	260.0 µL	522.5 µL

Step 4: T4 DNA Polymerase Master Mix

PROCEDURE

NOTE **Refer to Table 3.4 for the T4 DNA Polymerase Cocktail composition.**

IMPORTANT **The T4 DNA polymerase buffer supplied in the GeneChip® HT One-Cycle cDNA Synthesis Kit is 5X. First dilute 5X T4 DNA Polymerase Buffer to 1X buffer.**

1. Dilute the 5X T4 DNA Polymerase Buffer to a 1X concentration with RNase-free water.
2. Obtain an RNase-free 1.5 mL microfuge tube and label as "T4 DNA Pol."
3. Obtain a Bio-Rad Low-Profile PCR Tube Strip and label as "T4."
4. Add the components listed in Table 3.4 to the microfuge tube and mix well.
5. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the PCR strip tube.
6. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
7. Load the strip tube to column 4 of the cold block.

Table 3.4
T4 DNA Polymerase Cocktail for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
T4 DNA Polymerase	2.0 µL	69.3 µL	118.0 µL	236.0 µL
1X T4 DNA Polymerase Buffer	2.0 µL	69.3 µL	118.0 µL	236.0 µL
Total Volume	4.0 µL	138.6 µL	236.0 µL	472.0 µL
Volume per Strip Tube Well		16.0 µL	27.8 µL	57.0 µL

Step 5: IVT Master Mix

PROCEDURE

NOTE **Refer to Table 3.5 for the IVT Cocktail Master Mix composition.**

1. Obtain a 5 mL BD Falcon Test Tube with a clip-on cap for large volumes or a 1.5 mL microfuge tube for smaller volumes (for 32 reactions or less) and label as "IVT."
2. Obtain an 0.85 mL QIAGEN Elution Strip Tube and label as "IVT."
3. Add the components listed in Table 3.5 to the BD Falcon Test Tube or the microfuge tube and mix well.
4. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the QIAGEN Elution Strip Tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tube to column 5 of the cold block.

Table 3.5
IVT Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
10X IVT Buffer	6.0 µL	168.0 µL	336.0 µL	672.0 µL
IVT Labeling NTP Mix	18.0 µL	504.0 µL	1,008.0 µL	2,016.0 µL
IVT Labeling Enzyme Mix	6.0 µL	168.0 µL	336.0 µL	672.0 µL
T7 RNA Polymerase	1.0 µL	28.0 µL	56.0 µL	112.0 µL
Nuclease-free Water	7.0 µL	196.0 µL	392.0 µL	784.0 µL
Total Volume	38.0 µL	1,064.0 µL	2,128.0 µL	4,256.0 µL
Volume per Strip Tube Well		131.0 µL	264.0 µL	529.0 µL

Step 6: Fragmentation Buffer

PROCEDURE

NOTE **Refer to Table 3.6 for the Fragmentation Cocktail composition.**

1. Obtain a Bio-Rad Low-Profile PCR Tube Strip and label as "Frag."
2. Aliquot the volume listed in Table 3.6 into each well of the strip tube.
3. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
4. Load the strip tube to column 6 of the cold block.

Table 3.6
Fragmentation Cocktail for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
5X Fragmentation Buffer (Volume per Strip Tube Well)	7.5 µL	26.7 µL	53.4 µL	106.9 µL

Step 7: Hybridization Master Mix

These recipes take into account that it is necessary to make extra hybridization cocktail due to a small loss of volume (10-20 µL) during each hybridization.

REAGENT PREPARATION

12X MES Stock Buffer

(1.22 M MES, 0.89 M [Na^+])

For 1,000 mL:

64.61 g of MES Hydrate

193.3 g of MES Sodium Salt

800 mL of Molecular Biology Grade water

Mix and adjust volume to 1,000 mL.

The pH should be between 6.5 and 6.7. Filter through a 0.2 µm filter.

IMPORTANT !

Do not autoclave. Store at 2°C to 8°C, and shield from light. Discard solution if yellow.

2X Hybridization Buffer

(Final 1X concentration is 100 mM MES, 1 M [Na^+], 20 mM EDTA, 0.01% Tween-20)

For 50 mL:

8.3 mL of 12X MES Stock Buffer

17.7 mL of 5 M NaCl

4.0 mL of 0.5 M EDTA

0.1 mL of 10% Tween-20

19.9 mL of water

Store at 2°C to 8°C, and shield from light

PROCEDURE

IMPORTANT **If using the GeneChip® HT IVT Labeling Kit to prepare the target, a final concentration of 10% DMSO needs to be added in the hybridization cocktail for optimal results.**

NOTE **Refer to Table 3.7 for the Hybridization Cocktail Master Mix composition.**

1. Obtain a 14 mL BD Falcon Test Tube or a 50 mL centrifuge tube for larger volumes and label as "Hyb Mix."
2. Obtain one to six 0.85 mL QIAGEN Elution Strip Tubes and label as "Hyb."
3. Referring to Table 3.7, combine the components as indicated in a 14 mL BD Falcon Test Tube or a 50 mL centrifuge tube, depending on the total volume.
4. Aliquot the appropriate volumes of the master mix as indicated in Table 3.7 into each well of the QIAGEN Elution Strip Tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tubes to columns 7-12 of the cold block. The number of strip tubes will vary depending on the number of reactions.

Table 3.7
Hybridization Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
3 nM B2 Oligo	4.95 µL	133.7 µL	267.3 µL	534.6 µL
20X *BioB, C, D, Cre* (controls)	15.0 µL	405.0 µL	810.0 µL	1,620.0 µL
HS DNA (10 mg/mL)	3.0 µL	81.0 µL	162.0 µL	324.0 µL
Acetylated BSA (50 mg/mL)	3.0 µL	81.0 µL	162.0 µL	324.0 µL
2X Hyb Buffer	150.0 µL	4,050.0 µL	8,100.0 µL	16,200.0 µL
DMSO (100%)	30.0 µL	810.0 µL	1,620.0 µL	3,240.0 µL
Nuclease-free Water	64.05 µL	1,729.4 µL	3,458.7 µL	6,917.4 µL
Total Volume	270.0 µL	7,290.1 µL	14,580.0 µL	29,160.0 µL
# of QIAGEN Strips Used		2	3	6
Volume per Well in 1st Strip		600 µL	600 µL	600 µL
Volume per Well in 2nd Strip		300 µL	600 µL	600 µL
Volume per Well in 3rd Strip			600 µL	600 µL
Volume per Well in 4th Strip				600 µL
Volume per Well in 5th Strip				600 µL
Volume per Well in 6th Strip				600 µL

IMPORTANT ! **It is imperative that frozen stocks of 20X GeneChip® Eukaryotic Hybridization Controls are heated to 65°C for 5 minutes to completely resuspend the cRNA before aliquotting.**

Additional Reagent Preparation Steps Required

SETUP 75% EtOH RESERVOIR

Materials Needed

- E&K Scientific 96 Well Reservoir, High Profile 300 mL
- Phenix Universal Lid for 96-well plates

Procedure

1. Prepare 250 mL 75% EtOH and pour into reagent reservoir.
2. Cover the reservoir with a lid.

SETUP H_2O RESERVOIR

Materials Needed

- E&K Scientific 96 Well Reservoir, High Profile 300 mL
- Phenix Universal Lid for 96-well plates
- Nuclease-free Water

Procedure

1. Pour 250 mL nuclease-free water into the reagent reservoir.
2. Cover the reservoir with a lid.

cDNA AND cRNA PURIFICATION PREPARATION

Materials Needed

- Agencourt RNAClean™
- ABGene 1.2 mL Square Well Storage Plate, Low Profile
- Phenix Universal Lid for 96-well plates

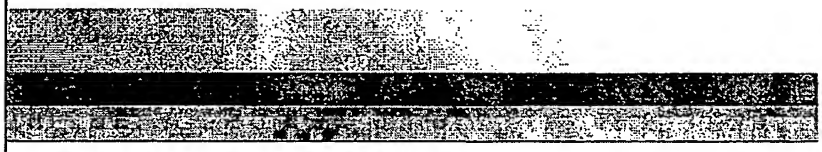
Procedure

1. Thoroughly shake the bottle of RNAClean™ solution before aliquoting 450 µL into each well of a 1.2 mL low profile reservoir.
2. Cover the reservoir with a lid.

Chapter 4

Array Station Setup and Target Preparation

Chapter 4



Introduction

This chapter describes a typical Array Station run for cartridge arrays. A schematic of the automated target preparation protocol is provided at the beginning to outline the steps in the procedure (Figure 4.1 to Figure 4.11).

This chapter also describes the procedures for using the iLink™ software, setting up the Array Station deck, and performing quantitation and normalization of cRNA.

Automated Target Preparation Schematic

The following outlines a complete sample preparation protocol.

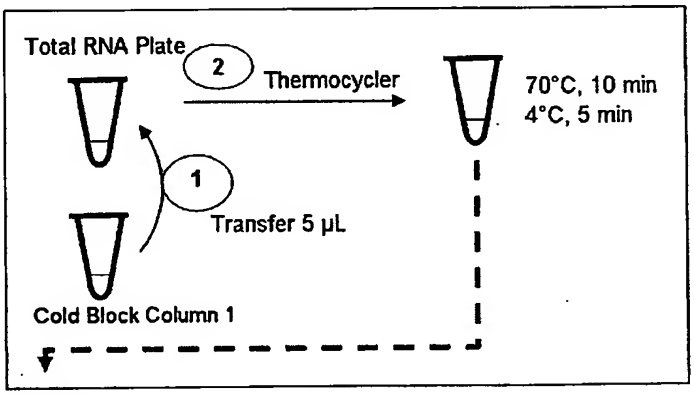


Figure 4.1
Step 1 & 2: Primer Anneal

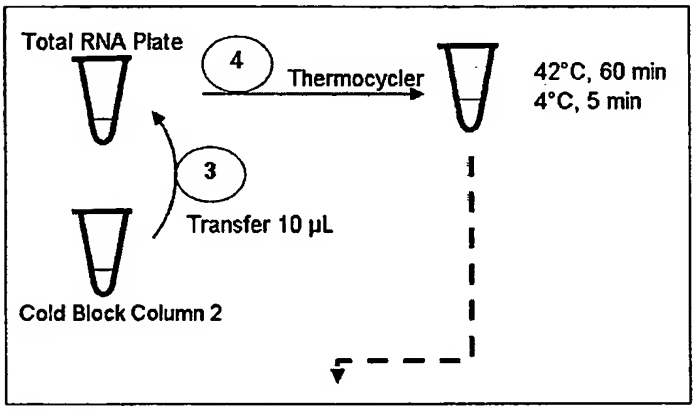


Figure 4.2
Step 3 & 4: First Strand cDNA Synthesis

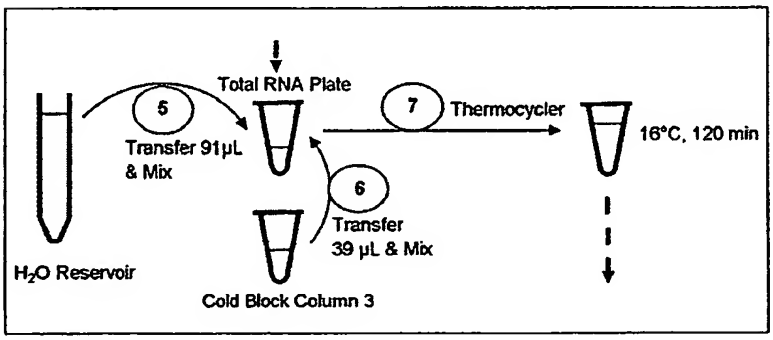


Figure 4.3
Steps 5, 6, and 7

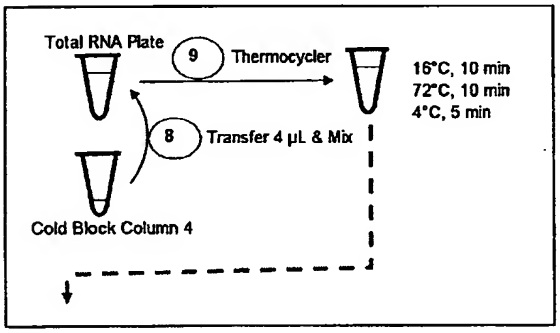


Figure 4.4
Step 8 & 9: T4 Polymerase Reaction

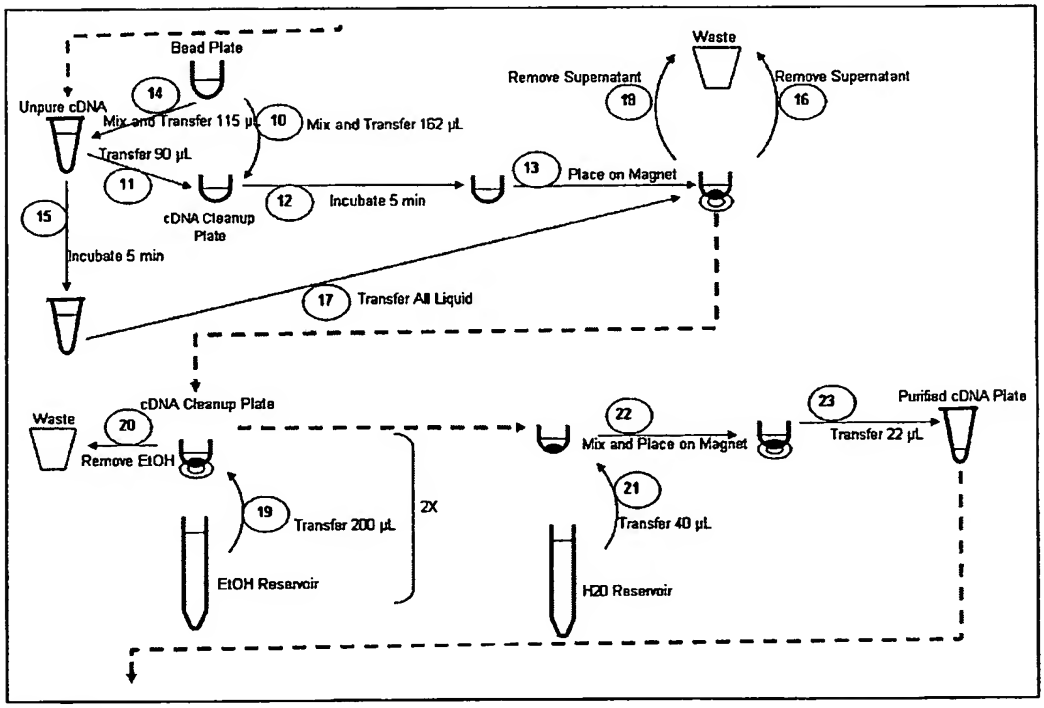


Figure 4.5
Steps 10 to 23: cDNA Clean-up and Elution (Purification)

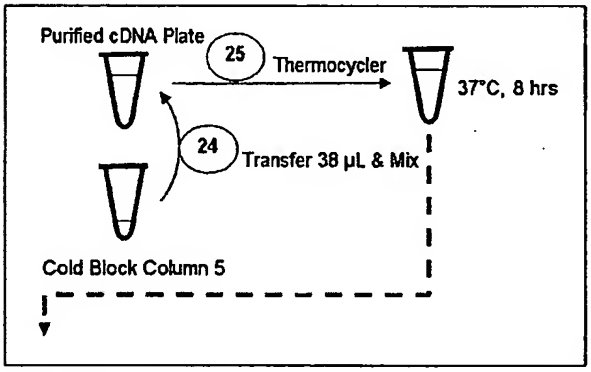


Figure 4.6
Steps 24 & 25: IVT Reaction

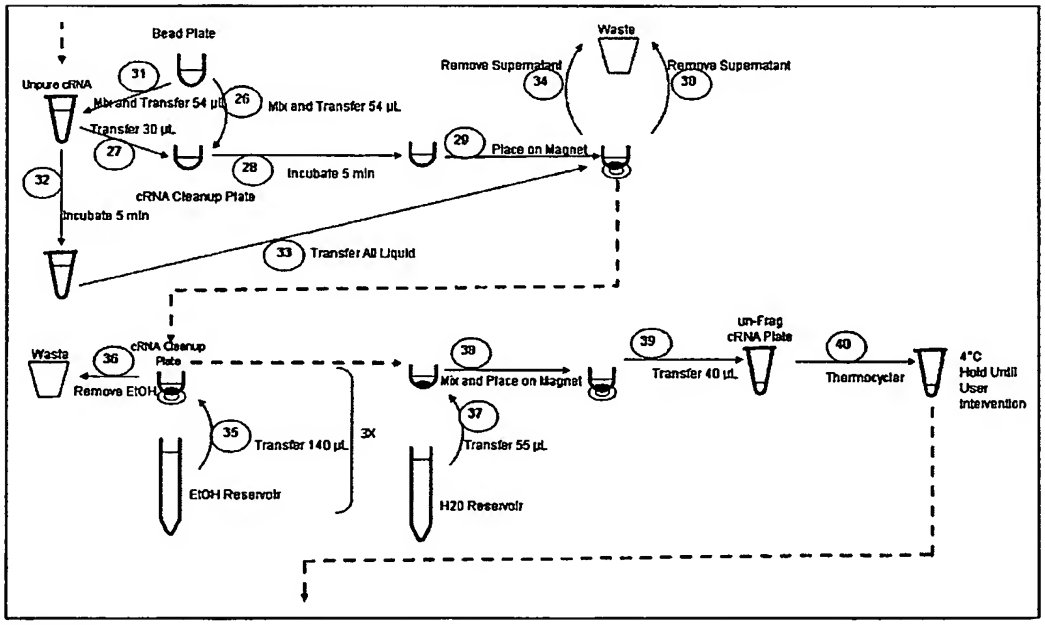


Figure 4.7
Steps 26 to 40: IVT Clean-up and Elution (Purification)

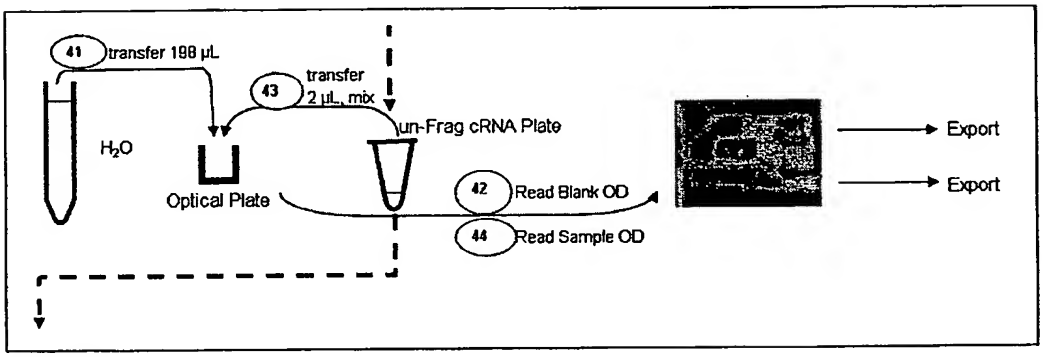


Figure 4.8
Steps 41 to 44: First Quantitation

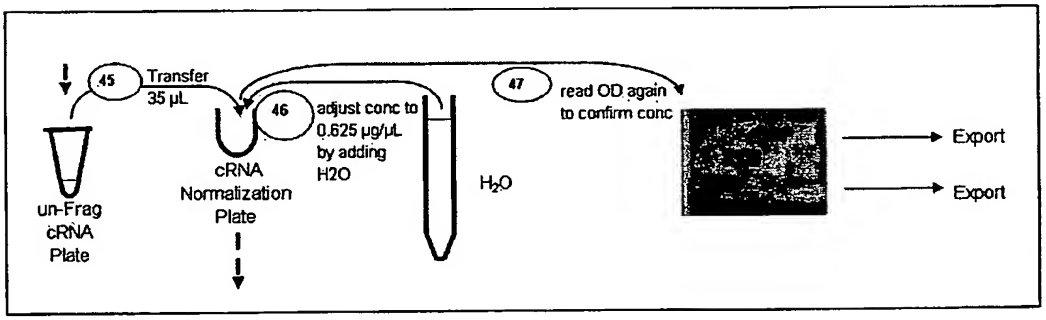


Figure 4.9
Steps 45 & 46 & 47: Normalization and Second Quantitation

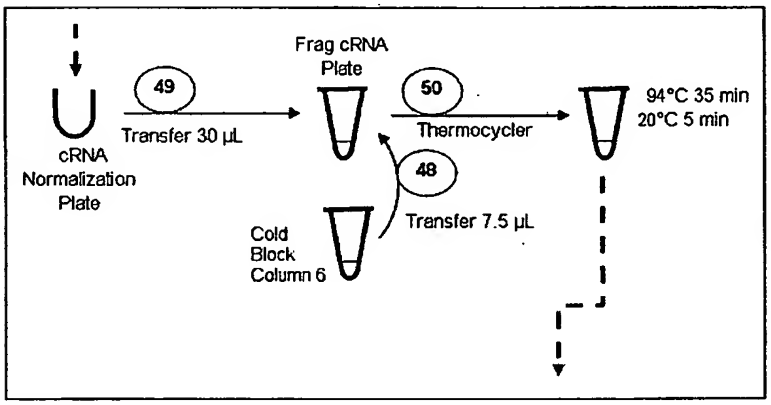


Figure 4.10
Steps 48 & 49 & 50: Fragmentation

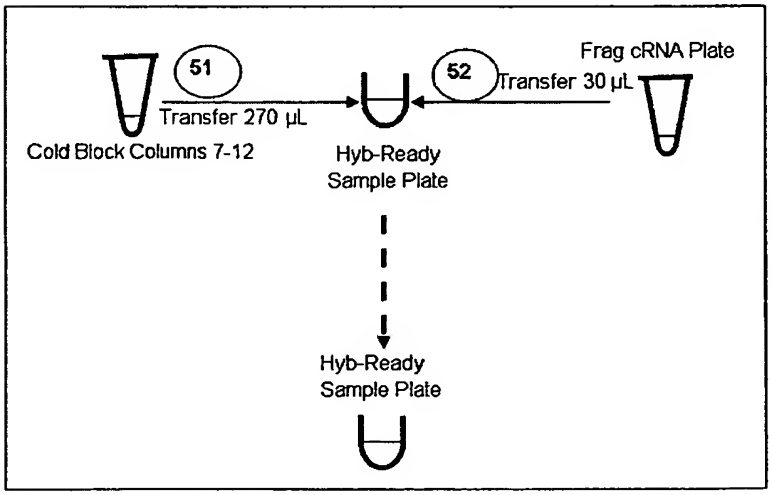


Figure 4.11
Steps 51 & 52: Hyb-sample Mix Transfer

Check List Before a Run

Before beginning a sample preparation run, you must make the following checks of the system.

1. Ensure that the water supply connections and waste water drainage are properly installed. In addition, the bottles (illustrated in Figure 4.12) must be filled with Molecular Biology Grade Water and the liquid waste container (illustrated in Figure 4.13) must be empty.

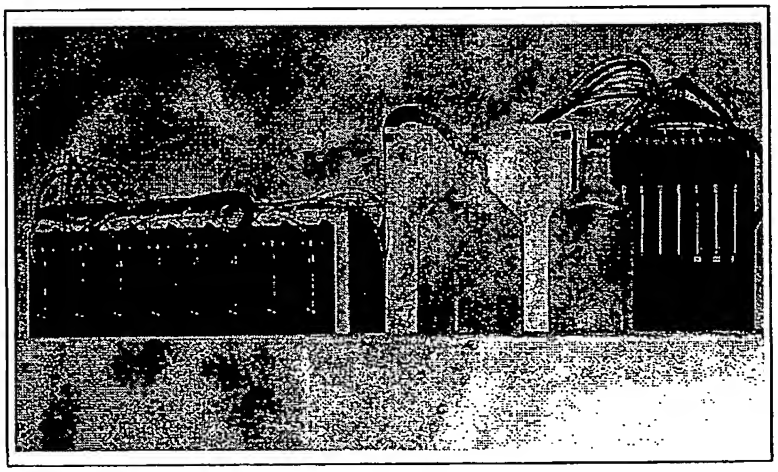


Figure 4.12
Tubing lines for water supply

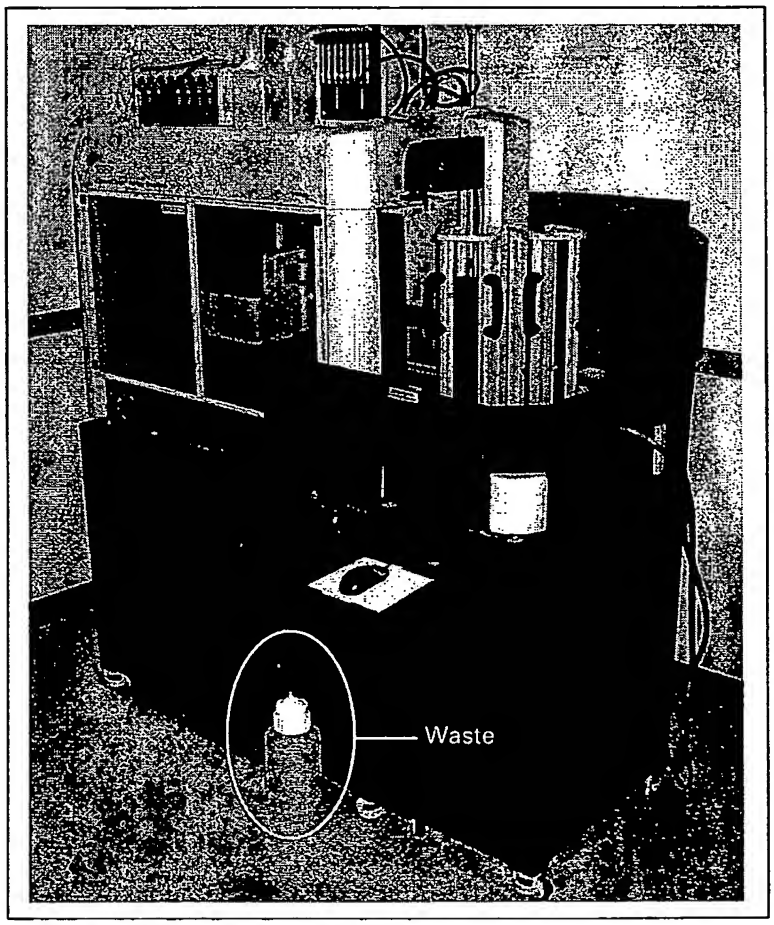


Figure 4.13
Liquid waste drainage

2. Ensure that all the pipette tip boxes are oriented correctly, fit snugly into the holders, and the holders fit into the Twister II Rack.

   Figure 4.14 to Figure 4.15 illustrate how to load the pipette tips into the Twister II Rack.

CAUTION

Tip Loading Requirement — The number of tip boxes needed for each run may vary depending on the deck layout used and the number of samples processed. Refer to Table 4.1 to determine the number of tip boxes to load.

Clean out empty tip boxes in Twister II® Rack 2.

Do not remove tip boxes while run is in progress.

Tip boxes should not extend above the top of the Rack.

Table 4.1
Pipette Tip Usage

Number of Rxns	8	16	24	32	40	48	56	64	72	80	88	96
Number of Tip Boxes Needed	2	4	6	8	10	11	14	15	17	19	22	22

3. Controlling static electrical interference: Static attraction can cause pipette tips to cling to the heads and to each other. To control static attraction, use non-sterile, RNase/DNase free tips and use a static gun to remove static discharge. See Figure 4.16 and Figure 4.17.

Figure 4.14
Loading the pipette tips into the holder and into the Rack

Figure 4.15
Loading the pipette boxes into the Rack

Figure 4.16
Removing static discharge from tip boxes with a static gun

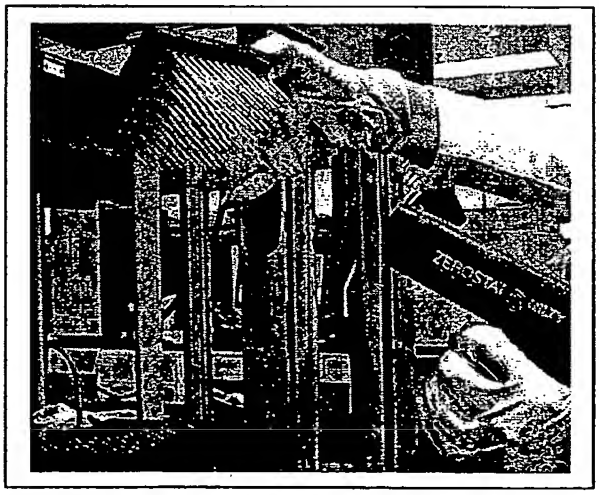


Figure 4.17
Removing static discharge from tips with a static gun

CLEAN THE BIO-RAD 96-WELL HARD-SHELL PCR PLATE LIDS

NOTE **The disposable pad under the arched lids should be cleaned before every run.**

Material required

- Ambion DNAZap™
- Ambion RNaseZap® Wipes

Procedure

1. Rinse the pad with DI water.
2. Wipe the pad with RNaseZap.
3. Rinse the pad with DI water.
4. Wipe the pad with DNAZap.
5. Thoroughly rinse the pad with DI water.
6. Dry the pad with pressurized clean air or nitrogen.

Beginning a Run — First Layout

This section shows you how to set up the deck and use the software to begin a sample preparation run for the first deck layout. The first layout protocol completes the following steps.

1. Primer anneal
2. First strand cDNA synthesis
3. Second strand cDNA synthesis
4. T4 polymerase synthesis
5. cDNA purification, wash and cDNA elution
6. IVT reaction
7. cRNA Cleanup and Elution
8. Pause for deck change to the second deck layout

PROCEDURE

1. Set up the deck with the appropriate consumables. Refer to Figure 4.18.
2. Assemble the Peltier adaptor and prechilled cold block. Ensure that the unit is securely tightened. Refer to Figure 4.19 and Figure 4.20.
3. Turn on the Peltier to 4°C. Refer Figure 4.21.

IMPORTANT !

The prechilled cold block and Peltier adaptor are assembled prior to the addition of the reagent strip tubes. It is important that the adaptor be at room temperature when joining with the cold block as it is difficult to fit the cold block flush against the adaptor if the adaptor is cold.

4. Load the reagent strip tubes onto the assembled cold block and adaptor. Refer to Figure 4.22.
5. Ensure that the tabs on the strip tubes are correctly seated in order to place the lid on the cold block.

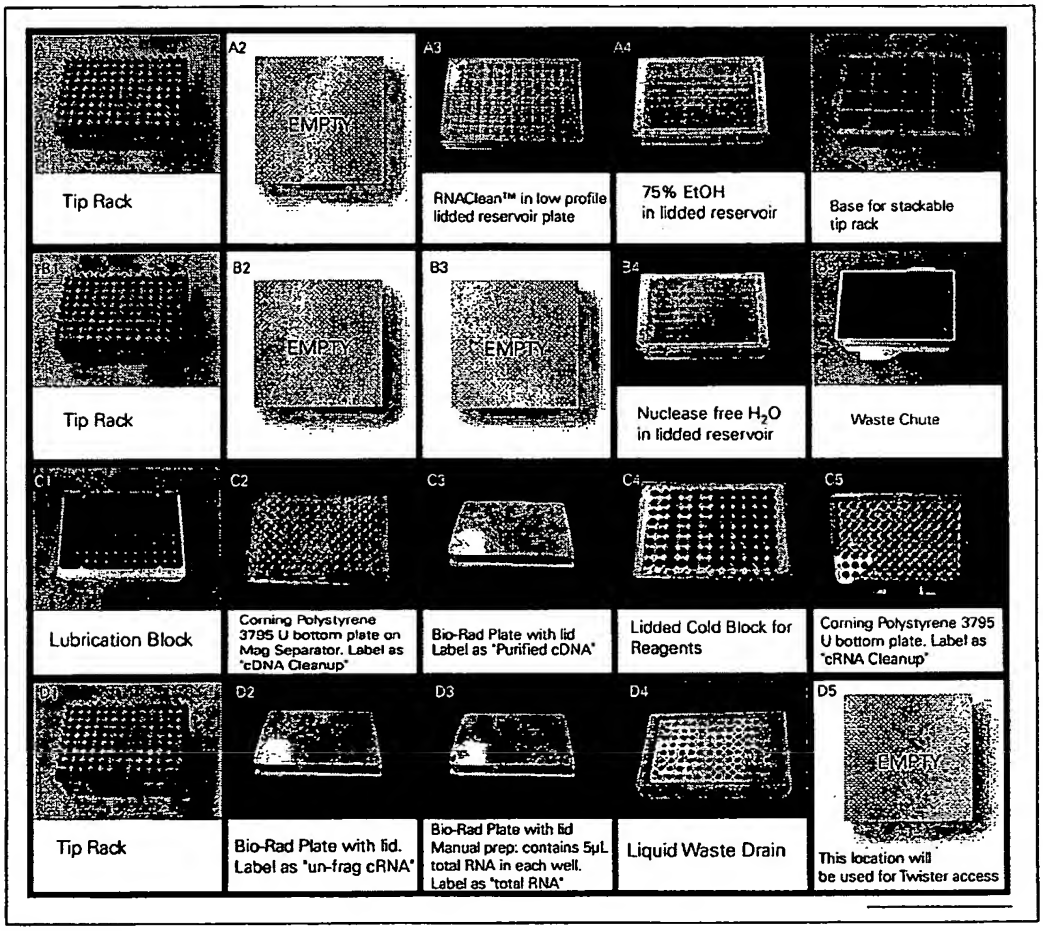


Figure 4.18
First deck layout.
Empty Rack 2, leaving the tip rack base there.
Refer to table Table 4.1 on page 51 to determine the number of tip boxes to load.

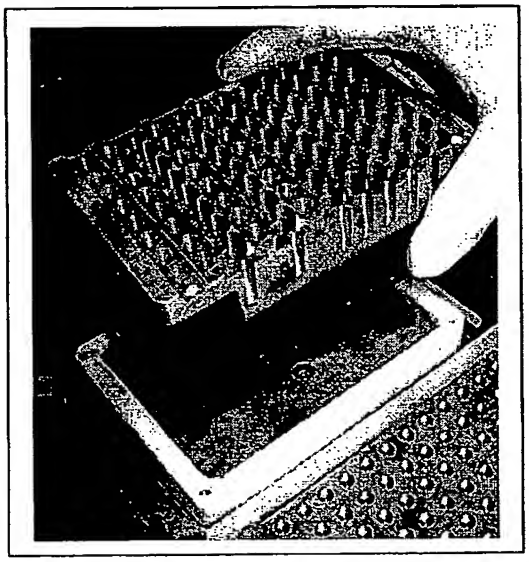


Figure 4.19
Loading the cold block on to the deck fixture

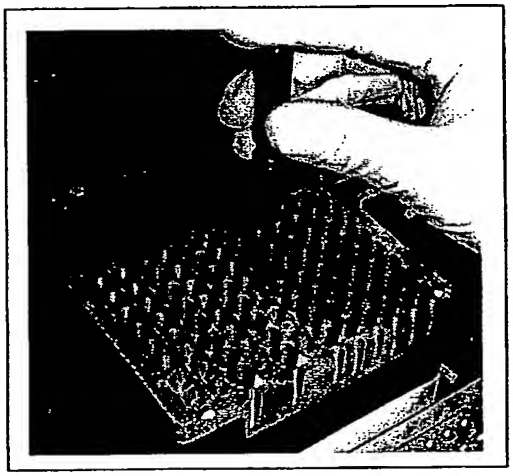


Figure 4.20
Securing the cold block

Figure 4.21
Setting the Peltier to 4°C

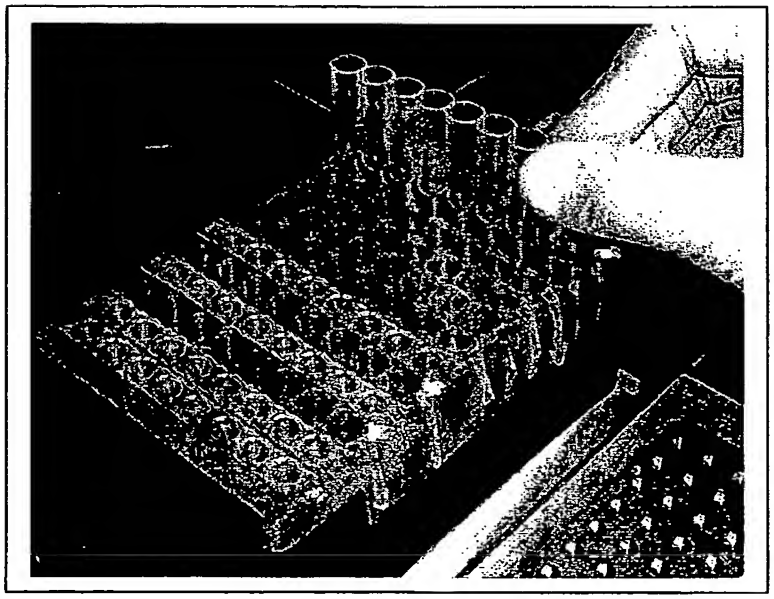


Figure 4.22
Loading the strips into the cold block

RUNNING AFFYMETRIX® GENE EXPRESSION TARGET PREPARATION (TP) PROTOCOL ON THE GENECHIP® ARRAY STATION

1. Check to confirm that the deck and racks are correctly populated with wellplates, tips, etc. and that the cold block is loaded with the correct reagents.
2. Open iLink by double clicking on desktop icon, or click Start → Programs → Caliper Life Sciences → iLink → iLink (Figure 4.23).

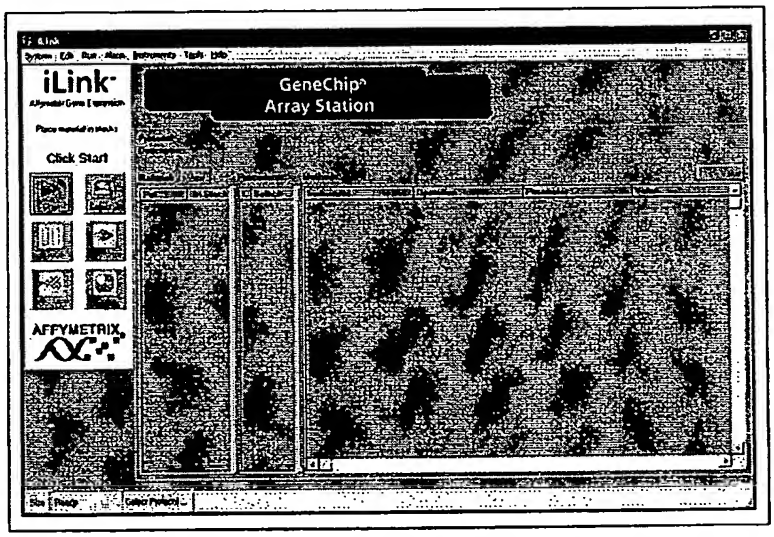


Figure 4.23

3. Click the Select button  .
   A protocol menu appears (Figure 4.24).

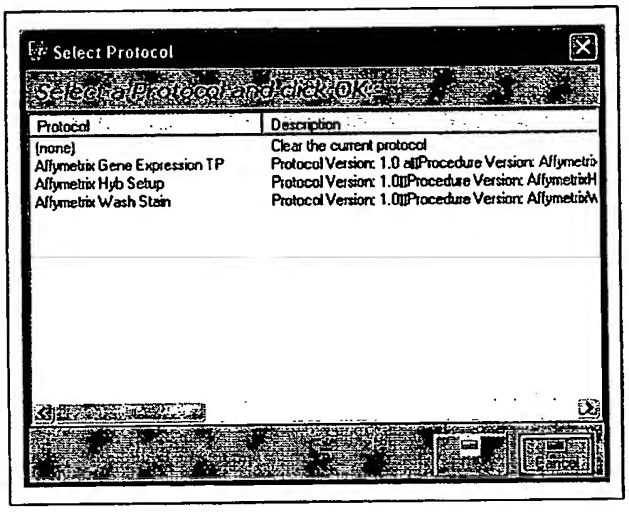


Figure 4.24

4. Select **Affymetrix Gene Expression TP** and click **OK**.

   The Eukaryotic one-cycle target labeling protocol is loaded into iLink (Figure 4.25).

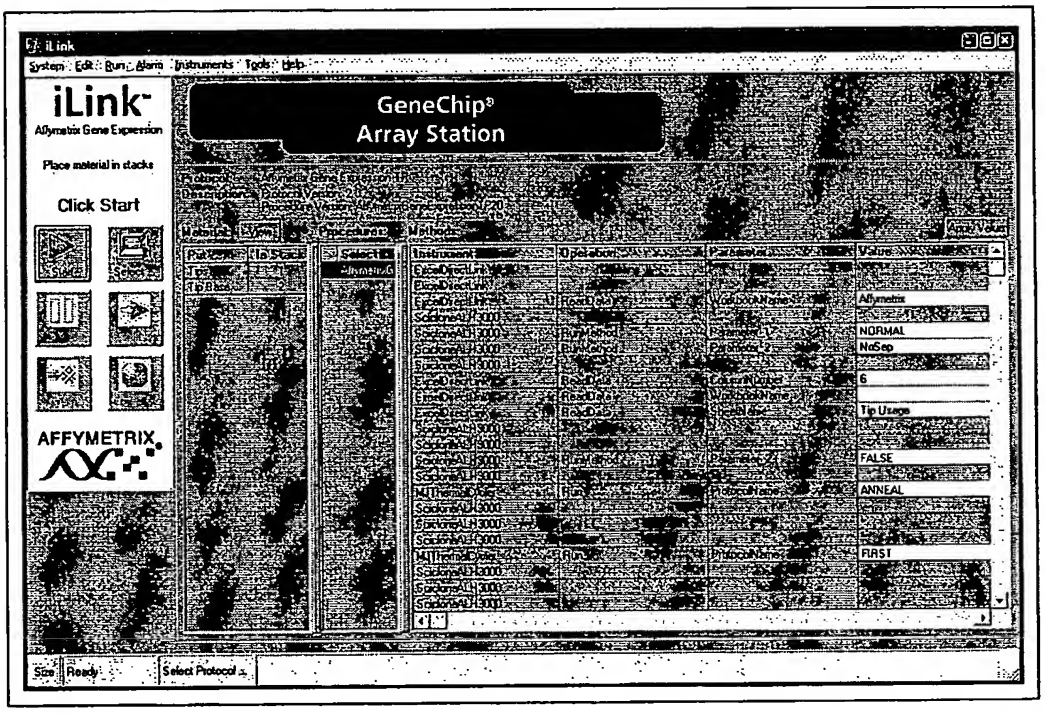


Figure 4.25

5. Click the Start button  to start the protocol.
   "Initializing Resources" appears as both the Caliper Sciclone and Twister II are initialized.

NOTE **Subsequent messages will appear in this space denoting the loading of software (Figure 4.26).**

The Affymetrix.xls user interface appears. Several run options may be selected.

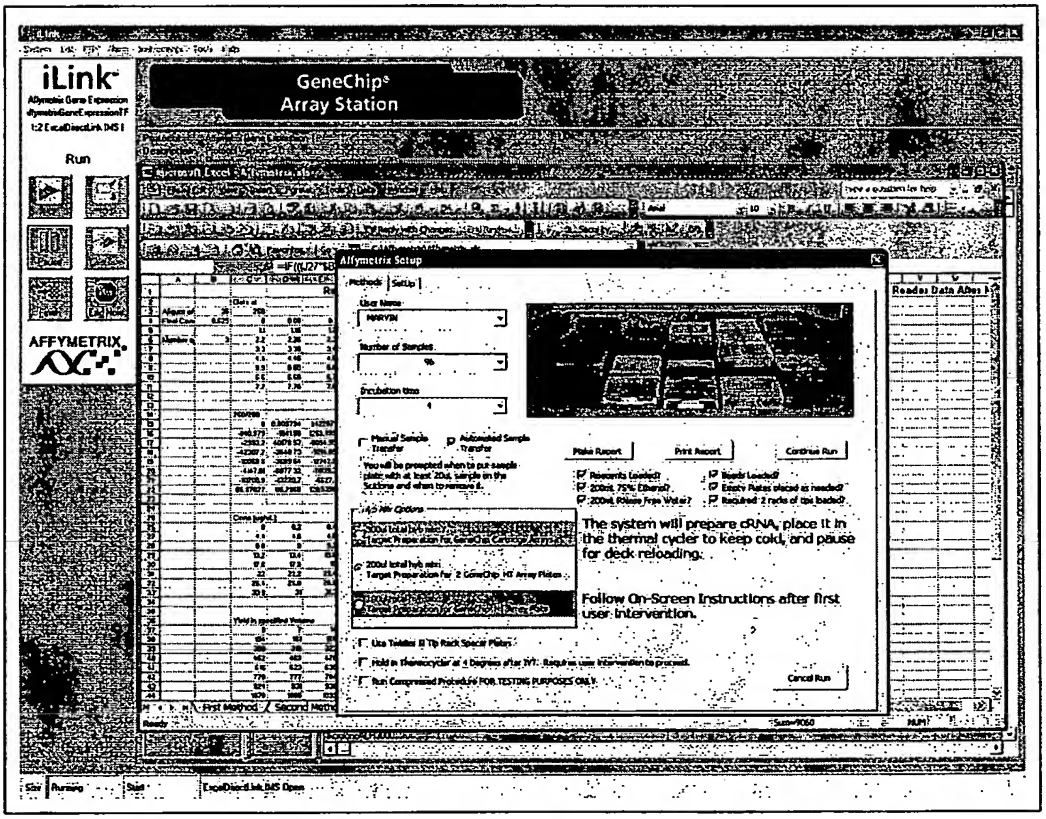


Figure 4.26

IMPORTANT !

The Array Station will prime the system before each run. During priming examine each syringe head to ensure there are no air bubbles. Remove bubbles by loosening each syringe, tap the lines to eliminate the bubbles, then retighten the screw.

6. Select the protocol parameters to enable the **Continue Run** button.

   A. From the drop-down option boxes, select User Name, Number of Samples by 8's (by column starting with position A1), and IVT Incubation Time.

   B. Select either **Manual Sample Transfer** or **Automated Sample Transfer**.

   If you select **Manual Sample Transfer** a message appears under the Manual Sample Transfer checkbox instructing you to load 5 µL of sample into the Bio-Rad Hard-Shell PCR Plate prior to starting the run (Figure 4.27).

   If you select **Automated Sample Transfer**, you will be prompted when to place and remove the plate as needed. Please refer to Appendix B for more information.

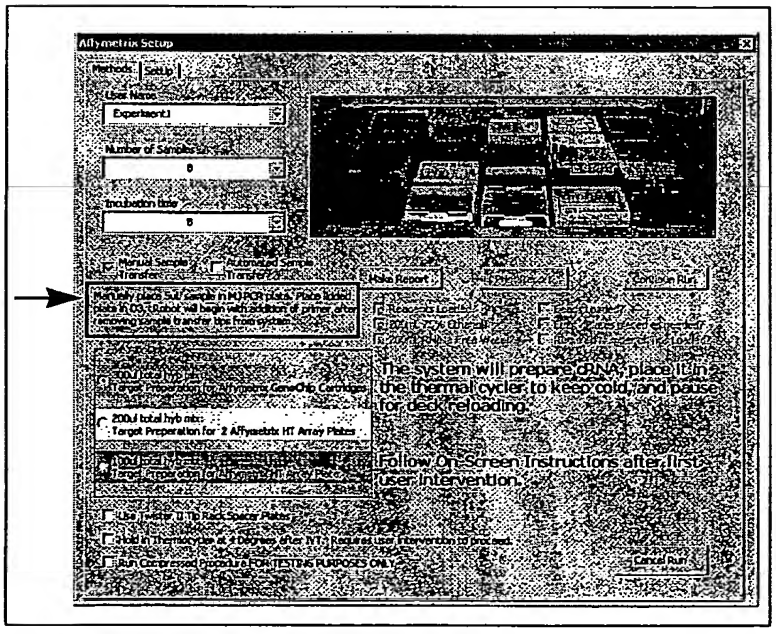


Figure 4.27

C. To make the final hybridization cocktail for the cartridge arrays, select the radio button for the 300 µL total hyb mix.

D. There are three options at the bottom of the Methods tab in the Affymetrix Setup Window:

1) Select the first option, "Use Twister II Tip Rack Spacer Plates" when using the spacer plates if there is a static electricity concern.

2) Select the second option, "Hold in Thermal Cycler at 4 Degrees after IVT. Requires user intervention to proceed" to enable post IVT procedures to start at a designated time.

3) The third option, "Run Compressed Procedure FOR TESTING PURPOSES ONLY," is for testing purposes only and should not be selected.

E. When appropriate options are selected, click the Make Report button. Immediately, the six check boxes under Make Report are enabled.

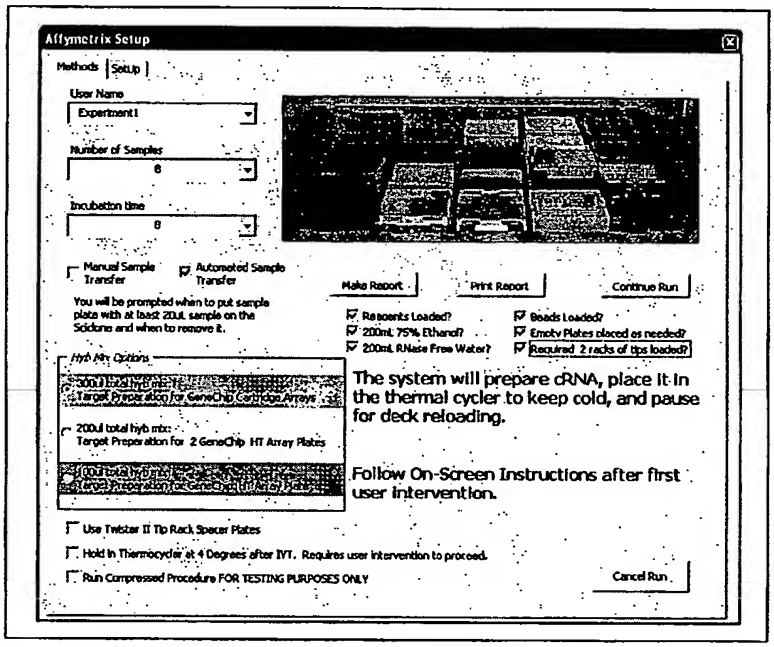


Figure 4.28
Affymetrix Setup Window

NOTE

To cancel before continuing the run follow these steps:

1. **Click the "End Now" button in iLink.**
2. **Click the "Cancel Run" button.**
3. **Two windows appear in succession.**
4. **Click "Yes" and "OK" sequentially to cancel the run.**

F. Use the six-option checklist on the right side of the window to ensure all reagents and consumables are loaded, and select each option (all six boxes should be checked as in Figure 4.28). The Continue Run button becomes enabled.

G. Click Continue Run.

A warning window appears asking if you are sure you want to run and the number of tip sets needed for the run (Figure 4.29).

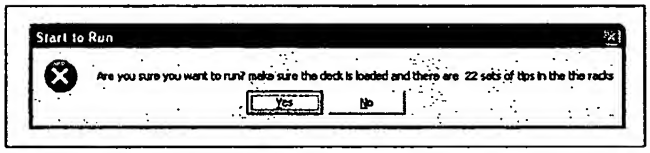


Figure 4.29

7. Verify that all aspects of the deck are prepared and there are sufficient tips in the Twister II Rack and click Yes.

   A. The selected options are recorded and Affymetrix.xls is saved as "user or expt name date_time stamp.xls." Refer to Figure 4.30.

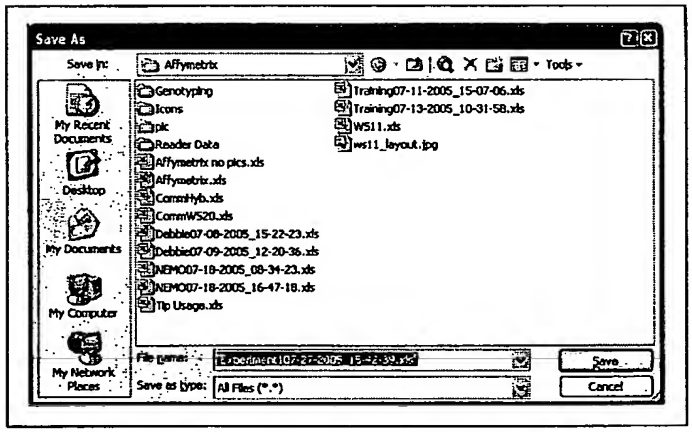


Figure 4.30

IMPORTANT **Do not change the File name. Do not edit, open or close any Excel file during the run.**

NOTE **The software loads a series of spreadsheets that provide information about the run conditions of the experiment. Please refer to Appendix C for further information.**

CAUTION **Do not close the spreadsheets or the Microsoft Excel program. Spreadsheets may be minimized.**

8. After clicking Continue Run, the options are read in from the Excel sheets; the Array Station TP application opens and the run commences (Figure 4.31).

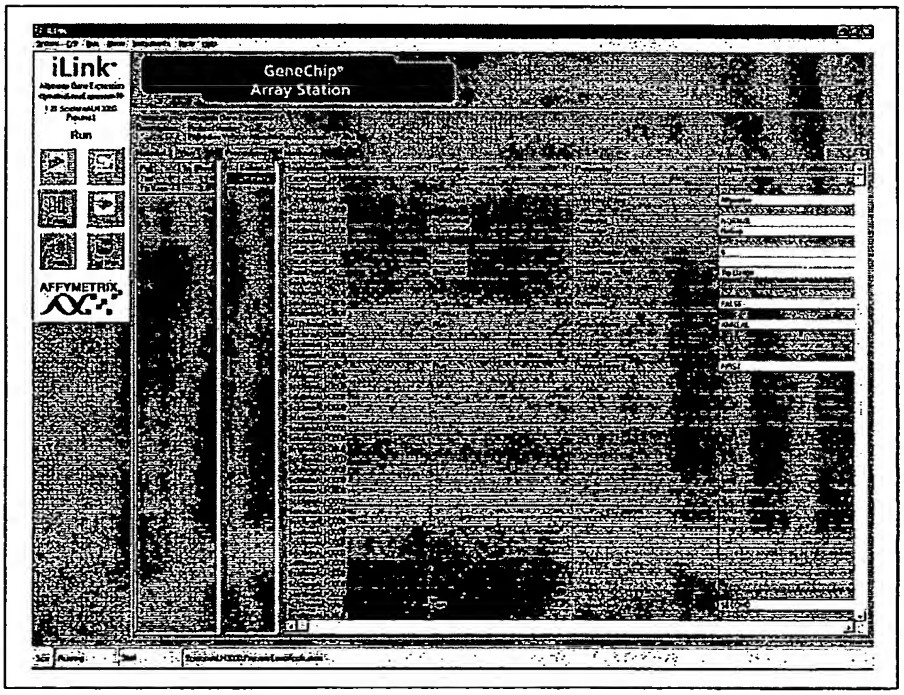


Figure 4.31
iLink™ starts the Target Preparation Protocol

NOTE

The starting sample plate's barcode is read automatically, for either the Bio-Rad 96-Well Hard-Shell PCR Plate (the "Manual Sample Transfer" option) or the round-bottom plate ("Automated Sample Transfer" option). Plates without barcodes will register as "No Response Detected" and the run will continue.

Barcode data will be copied to text files in the Barcodes folder by date and in the renamed Affymetrix.xls file.

9. If the option **Hold After IVT** was selected, the following message appears after the IVT run (Figure 4.32). An additional user intervention is required to proceed with cRNA purification. To continue select **OK** (Figure 4.32).

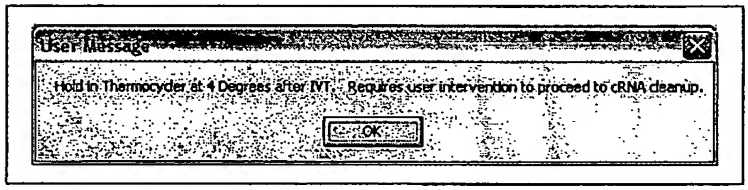


Figure 4.32

10. Continue to *Second Layout (User Intervention)* on page 69.

Second Layout (User Intervention)

This section shows you how to begin a sample preparation run for the second deck layout.

The second layout protocol completes the following steps:

1. First quantitation
2. Normalization
3. Second quantitation
4. Fragmentation
5. Preparation of hybridization-ready sample.

PROCEDURE

CAUTION **Do not click OK, as illustrated in the User Message in Figure 4.33, until the deck layout is changed. See Figure 4.34 for an illustration of the deck layout.**

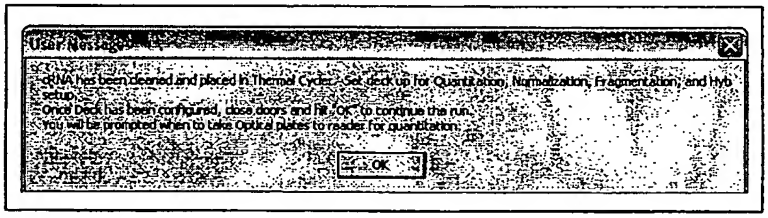


Figure 4.33

1. Change the deck layout as shown in Figure 4.34.
2. Follow the prompts on the workstation to complete this section of the protocol.

NOTE **After the completion of this section, the samples are ready for hybridization to the cartridge arrays.**

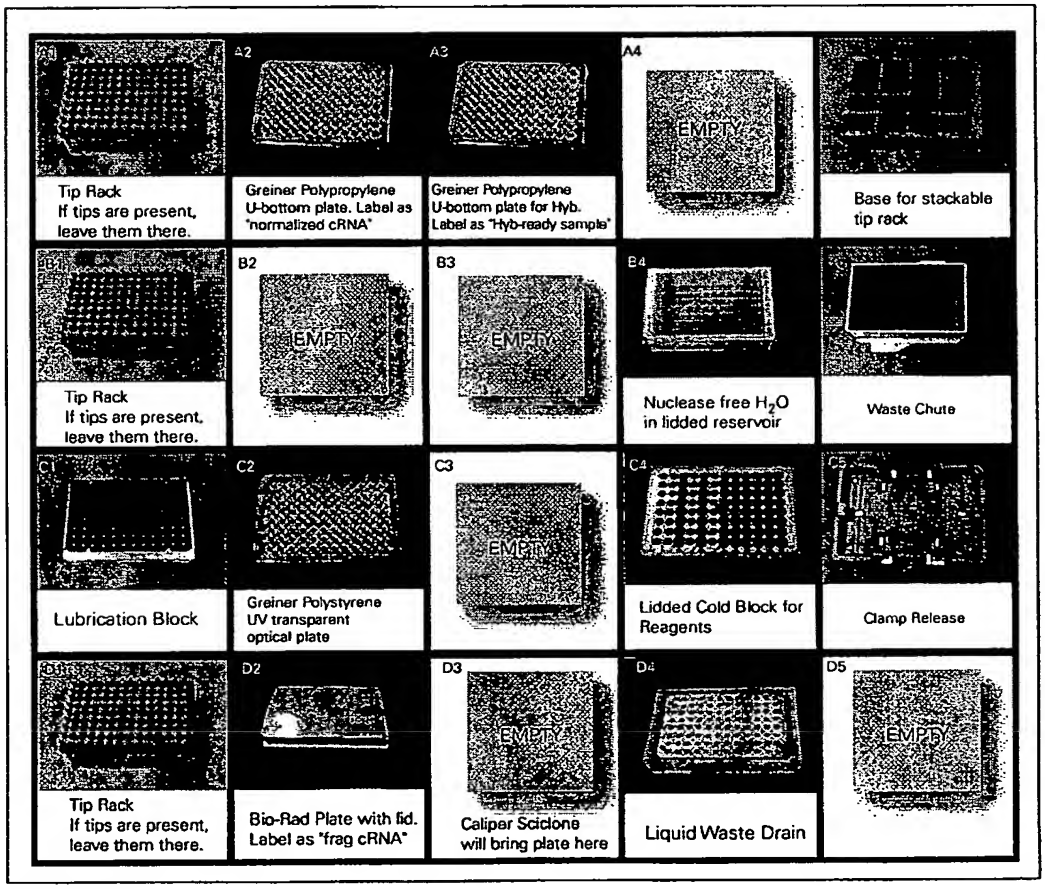


Figure 4.34
Second layout.
This requires user intervention to set up.

cRNA QUANTITATION AND NORMALIZATION

This section of the protocol details the user intervention steps necessary for calculating the yield of cRNA generated from the *in vitro* transcription reaction. The accurate calculation of the yield is necessary so that the correct amount of cRNA is added to the fragmentation reaction. Too much or too little cRNA added to the fragmentation reaction can result in incomplete or over fragmented cRNA and cause hybridization effects. This cRNA yield can be used as a check point to ensure that all the proceeding steps have been successfully completed and sufficient cRNA yield has been generated.

The GeneChip Array Station uses spectrophotometric analysis to determine the cRNA yield. The convention that 1 absorbance unit at 260 nm equals 40 µg/mL RNA is used.

- The absorbance at 260 nm and 280 nm is checked to determine sample concentration and purity.
- The A_{260}/A_{280} ratio is maintained close to 2.0 for pure RNA (ratios between 1.9 and 2.1 are acceptable).

NOTE **The following method assumes the use of the recommended spectrophotometer as part of the Array Station system. If a different spectrophotometer is used, refer to instructions in that user guide.**

cRNA FIRST QUANTITATION

1. Turn on the spectrophotometer. Before proceeding, ensure that the LCD screen on the spectrophotometer indicates that the UV lamp has warmed up successfully. Confirm that you have selected the proper wavelengths (260 and 280).
2. Start the SoftMax® Pro software. Either click the SoftMax Pro icon on the desktop or click on Start **All Programs** → **SoftMax Pro** → **SoftMax Pro**.

NOTE **The robot will remove the reaction plate from the thermal cycler and put 198 µL of water into the optical plate.**

CAUTION **When the User Message appears, as illustrated in Figure 4.35, do not click "OK" until Step 7.**

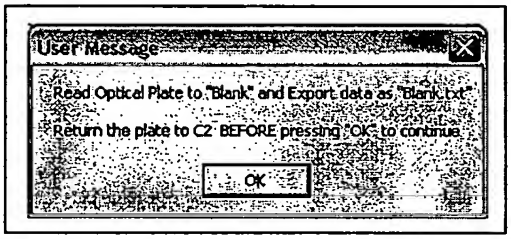


Figure 4.35

3. Remove the optical plate from position C2 on the deck and place the plate on the spectrophotometer tray.
4. Press the **Read** button in the SoftMax Pro software and make sure that the **Replace** option is selected.

   The spectrophotometer will read the tray as a blank.
5. Using the SoftMax Pro software, export the readings as a file called "Blank.txt" to the folder c:\Affymetrix\ReaderData. Also save the blank reading on floppy disc as a backup.

IMPORTANT **Use the export function to save the file in text format. If you are using other software, export or save data as a text file.**

Do not open the Excel file from task bar until the run is finished. Opening an Excel file during the run will interfere with data processing.

6. Go to c:\Affymetrix\ReaderData to check that the software has updated "Blank.txt" with the correct date and time.
7. Return the plate on the deck to position C2 and click OK.

NOTE **The Array Station adds 2 µL of sample to 198 µL of water in the optical plate.**

CAUTION **When the User Message appears, as illustrated in Figure 4.36, do not click "OK" until Step 2 of Normalization on page 75.**

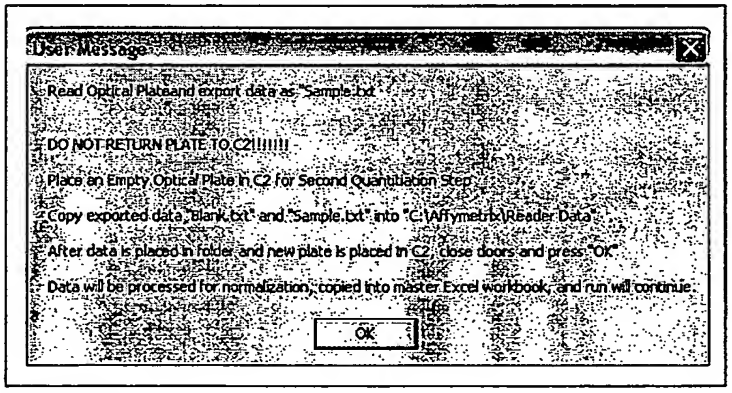


Figure 4.36

8. Remove the optical plate and place in the spectrophotometer.
9. Open the SoftMax Pro software.

10. Press the **Read** button in the SoftMax Pro software and ensure that you have selected the **Replace** option.

    The spectrophotometer will now read the plate.

11. When the spectrophotometer completes the optical read, export the data as "sample.txt" to the folder c:\Affymetrix\ReaderData. Save the sample data on a floppy disc as a backup.

12. Go to c:\Affymetrix\ReaderData to check that the software has updated "Sample.txt" with the correct date and time.

13. Discard optical plate.

NORMALIZATION

1. Place a clean optical plate on the Array Station deck at position C2.
2. When the User Message appears, as illustrated in Figure 4.37, click OK.

IMPORTANT !

The software will automatically process and analyze the spectrophotometer data. The robot will add water to each well to make the dilution of cRNA ready for fragmentation. This process takes approximately 30 minutes to complete for a full 96 sample plate.

Following normalization, the Array Station will prepare a blank plate so that the normalized samples can be read to verify normalization. The blank plate will contain 198 µL of water in each well.

CAUTION

When the User Message appears, as illustrated in Figure 4.37, do not click "OK" until Step 6 of Second Quantitation on page 76.

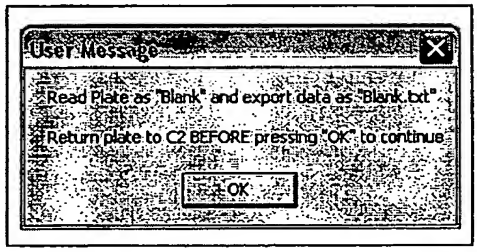


Figure 4.37

SECOND QUANTITATION

1. Remove the optical plate from position C2 and place the optical plate on the spectrophotometer tray.
2. Start the SoftMax Pro software. Either click the Softmax Pro icon on the desktop or click **Start All Programs** → **SoftMax Pro** → **SoftMax Pro**.
3. Press the **Read** button in the SoftMax Pro software and ensure that you have selected the **Replace** option.

   The spectrophotometer will now read the plate.
4. When the spectrophotometer completes the optical read, export the data in the appropriate format as "Blank.txt" to the folder c:\Affymetrix\ReaderData. Save the blank reading on a floppy disc as a backup.

NOTE **The procedure outlined in Step 4 will overwrite the previously exported Blank.txt file.**

5. Go to c:\Affymetrix\ReaderData, to ensure the "Blank.txt" file is updated with the correct date and time.
6. Replace the optical plate back on the deck in position C2 and click OK.

NOTE **The Array Station will now add 2 µL to the 198 µL of the water and will mix the sample with pipette mixes.**

CAUTION **When the User Message appears, as illustrated in Figure 4.38, do not click "OK" until Step 12.**

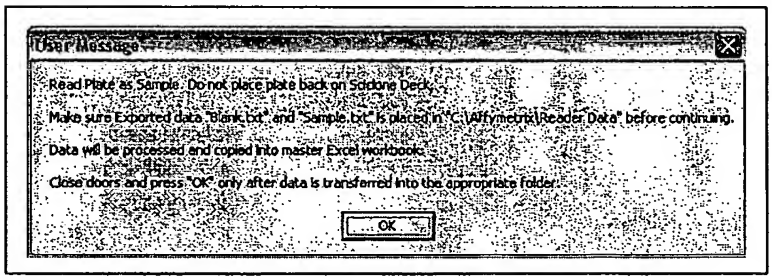


Figure 4.38

7. Remove the optical plate from position C2 on the Array Station and place in the spectrophotometer.
8. Press the **Read** button in the SoftMax Pro software make sure that the **Replace** option is selected.

   The spectrophotometer will now read the plate.
9. When the spectrophotometer completes the optical read, export the file in the appropriate format as "Sample.txt" to the folder c:\Affymetrix\ReaderData. Save the data on a floppy disc as a backup.
10. Go to c:\Affymetrix\ReaderData, ensure the software has updated "Sample.txt" with the correct date and time.
11. Exit the SoftMax Pro program.
12. Discard the optical plate and click OK.

NOTE

The Array Station will analyze the optical readings. The results will be copied to the Excel spreadsheet created at the beginning of the run. Normalization can be verified by inspecting the spreadsheet. Following the analysis, the sample preparation method will continue with the fragmentation step.

After the second quantitation, the samples should be at a dilution around 0.625 µg/µL. If the dilution of the sample is below 0.625 µg/µL, the sample will not be normalized, but will continue through the method.

FRAGMENTATION AND HYBRIDIZATION COCKTAIL

After the second quantitation and cRNA yield calculation, the Array Station will continue to the fragmentation step and make the hybridization-ready sample without user intervention.These two processes take approximately two hours for a full 96 sample plate. After the run has completed, a message will appear as illustrated in Figure 4.39.

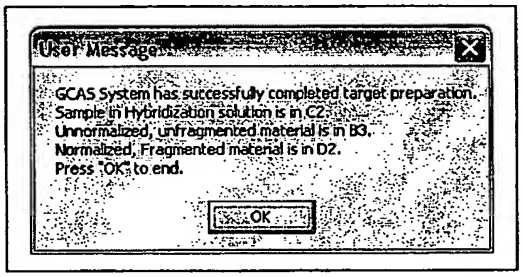


Figure 4.39
User Message indicating the completion of target preparation.

1. Manually transfer the hybridization ready samples from the 96 well plate to 1.5 mL RNase-free microcentrifuge tubes to continue to hybridization or for storage.

GeneChip® Array Station Clean Up

After completion of the target preparation, follow the steps below to cleanup the Array Station.

1. Cover the unfragmented and fragmented RNA with an adhesive plate sealer. Use the plate roller to securely cover the sealer on the plate to prevent evaporation.
2. Store the sealed plates at –20°C.
3. Dispose of the EtOH, water, pipette tips, and waste water appropriately.
4. Wipe up any spills that may have occurred.
5. Close iLink, which in turn closes all subsequent applications.
6. Check and refill Z8 water reservoir level.

Chapter 5

Eukaryotic One-Cycle Target Hybridization for Cartridge Arrays

Chapter 5

Eukaryotic One-Cycle Target Hybridization

At this point in target preparation, a hybridization-ready sample is denatured and loaded into a cartridge array.

SUPPLIES

- Hybridization Oven 640: Affymetrix, P/N 800138 (110V) or 800139 (220V)
- Micropipettors, (P-2, P-20, P-200, P-1000): Rainin Pipetman (or equivalent)
- Sterile-barrier pipette tips and non-barrier pipette tips
- Heatblock

PROCEDURE

1. Equilibrate probe array to room temperature immediately before use.

NOTE **It is important to allow the arrays to equilibrate to room temperature completely. Specifically, if the rubber septa are not equilibrated to room temperature, they may be prone to cracking, which can lead to leaks.**

2. Heat the hybridization cocktail to 99°C in a heat block for 5 minutes.
3. Meanwhile, wet the array by filling it through one of the septa (see Figure 5.1 for location of the probe array septa) with appropriate volume of 1X Hybridization Buffer using a micropipettor and appropriate tips. See Table 5.1 for hybridization and fill volumes of the various array sizes.

NOTE **To make a 1X Hybridization Buffer, make a 1:1 dilution of the 2X Hybridization Buffer.**

NOTE **It is necessary to use two pipette tips when filling the probe array cartridge: one for filling and the second to allow venting of air from the hybridization chamber.**

4. Using the Hybridization Oven, incubate the probe array filled with 1X Hybridization Buffer at 45°C and rotate at 60 rpm for 10 minutes.

Table 5.1
Probe Array Cartridge Volumes

Array	Hybridization Volume	Total Fill Volume
49 Format (Standard)	200 µL	250 µL
64 Format	200 µL	250 µL
100 Format (Midi)	130 µL	160 µL
169 Format (Mini)	80 µL	100 µL
400 Format (Micro)	80 µL	100 µL

5. Transfer the hybridization cocktail that has been heated at 99°C, in step 2, to a 45°C heat block for 5 minutes.
6. Remove the buffer solution from the probe array cartridge and fill with appropriate volume (Table 5.1) of the clarified hybridization cocktail, avoiding any insoluble matter at the bottom of the tube.
7. Place probe array into the Hybridization Oven, already set to 45°C.

   Avoid stress to the motor; load probe arrays in a balanced configuration around the axis. Rotate at 60 rpm.
8. Hybridize for 16 hours.

   During the latter part of the 16-hour hybridization, proceed to Chapter 6 to prepare reagents required immediately after completion of hybridization.

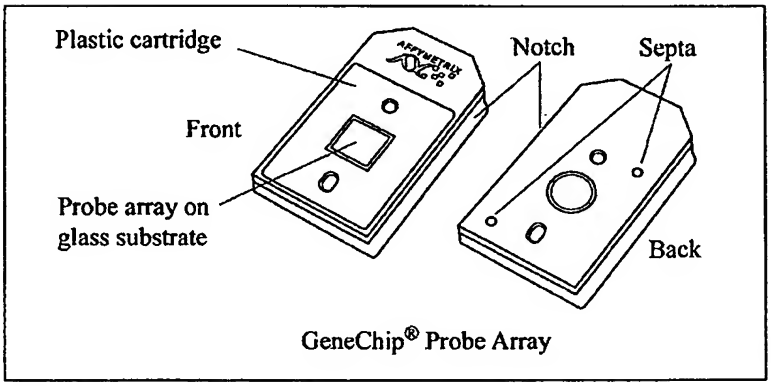


Figure 5.1
GeneChip® Probe Array

Chapter 6

Washing, Staining, and Scanning for Eukaryotic Cartridge Arrays

Chapter 6

Reagents and Materials Required

The following reagents and materials have been tested and evaluated by Affymetrix. Information and part numbers listed are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix D of this manual.

- DEPC-Treated Water: Ambion, P/N 9920
- 5 M NaCl, RNase-free, DNase-free: Ambion, P/N 9760G
- PBS, pH 7.2; Invitrogen Life Technologies: P/N 20012-027
- Distilled water: Invitrogen Life Technologies, P/N 15230-147
- Bovine Serum Albumin (BSA) solution (50 mg/mL): Invitrogen Life Technologies, P/N 15561-020
- R-Phycoerythrin Streptavidin: Molecular Probes, P/N S-866
- 20X SSPE (3 M NaCl, 0.2 M NaH_2PO_4, 0.02 M EDTA): Cambrex, P/N 51214
- Goat IgG, Reagent Grade: Sigma-Aldrich, P/N I 5256
- Anti-streptavidin antibody (goat), biotinylated: Vector Laboratories, P/N BA-0500
- Surfact-Amps® 20 (Tween-20), 10%: Pierce Chemical, P/N 28320

Miscellaneous Supplies

- Sterile, RNase-free, microcentrifuge vials, 1.5 mL: USA Scientific, P/N 1415-2600 (or equivalent)
- Tough-Spots®, Label Dots: USA Scientific, P/N 9185-0000
- Micropipettors, (P-2, P-20, P-200, P-1000): Rainin Pipetman® (or equivalent)
- Sterile-barrier pipette tips and non-barrier pipette tips

Reagent Preparation

Wash Buffer A: Non-Stringent Wash Buffer

(6X SSPE, 0.01% Tween-20)

For 1,000 mL:

300 mL of 20X SSPE

1.0 mL of 10% Tween-20

699 mL of water

Filter through a 0.2 µm filter

Wash Buffer B: Stringent Wash Buffer

(100 mM MES, 0.1 M [Na^+], 0.01% Tween-20)

For 1,000 mL:

83.3 mL of 12X MES Stock Buffer (see Chapter 5 for reagent preparation)

5.2 mL of 5 M NaCl

1.0 mL of 10% Tween-20

910.5 mL of water

Filter through a 0.2 µm filter

Store at 2°C to 8°C and shield from light

2X Stain Buffer

(Final 1X concentration: 100 mM MES, 1 M [Na^+], 0.05% Tween-20)

For 250 mL:

41.7 mL of 12X MES Stock Buffer (see Chapter 5 for reagent preparation)

92.5 mL of 5 M NaCl

2.5 mL of 10% Tween-20

113.3 mL of water

Filter through a 0.2 µm filter

Store at 2°C to 8°C and shield from light

10 mg/mL Goat IgG Stock

Resuspend 50 mg in 5 mL of 150 mM NaCl

Store at 4°C

NOTE **If a larger volume of the 10 mg/mL IgG stock is prepared, aliquot and store at –20°C until use. After the solution has been thawed it should be stored at 4°C. Avoid additional freezing and thawing.**

PREPARING THE STAINING REAGENTS

Prepare the following reagents. Volumes given are sufficient for one probe array.

SAPE Stain Solution

Streptavidin Phycoerythrin (SAPE) should be stored in the dark at 4°C, either foil-wrapped or kept in an amber tube. Remove SAPE from the refrigerator and tap the tube to mix well before preparing stain solution. Do not freeze SAPE. Always prepare the SAPE stain solution fresh, on the day of use.

Table 6.1
SAPE Solution Mix

Component	Volume	Final Concentration
2X Stain Buffer	600.0 µL	1X
50 mg/mL BSA	48.0 µL	2 mg/mL
1 mg/mL Streptavidin Phycoerythrin (SAPE)	12.0 µL	10 µg/mL
DI H_2O	540.0 µL	—
Total Volume	1,200 µL	

Mix well and divide into two aliquots of 600 µL each to be used for stains 1 and 3.

ANTIBODY SOLUTION

Table 6.2
Antibody Solution Mix

Components	Volume	Final Concentration
2X Stain Buffer	300.0 µL	1X
50 mg/mL BSA	24.0 µL	2 mg/mL
10 mg/mL Goat IgG Stock	6.0 µL	0.1 mg/mL
0.5 mg/mL biotinylated antibody	3.6 µL	3 µg/mL
DI H_20	266.4 µL	—
Total Volume	600 µL	

Entering Experiment Information

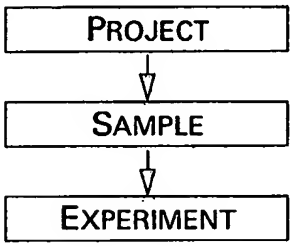
To wash, stain, and scan a probe array, an experiment must first be registered in GeneChip® Operating Software (GCOS). Please follow the instructions detailed in the *"Setting Up an Experiment"* section of the *Affymetrix GeneChip® Operating Software User's Guide* (P/N 701439).

The fields of information required for registering experiments in GCOS are:

- Sample Name
- Sample Type
- Project
- Experiment Name
- Probe Array Type

Sample templates, Experiment templates, and array barcodes can also be employed in GCOS to standardize and simplify the registration process. Please see the *GeneChip Operating Software User's Guide* for more information.

The Project, Sample Name, and Experiment Name fields establish a sample hierarchy that organizes GeneChip gene expression data in GCOS. In terms of the organizational structure, the Project is at the top of the hierarchy, followed by Sample Name, and then Experiment Name.



Preparing the Fluidics Station

The Fluidics Station 400, or 450/250 is used to wash and stain the cartridge arrays. It is operated using GCOS.

SETTING UP THE FLUIDICS STATION

1. Turn on the Fluidics Station using the toggle switch on the lower left side of the machine.
2. Select **Run** → **Fluidics** from the menu bar.

   The Fluidics Station dialog box appears with a drop-down list for selecting the experiment name for each of the fluidics station modules. A second drop-down list is accessed for choosing the Protocol for each of the fluidics station modules.

NOTE **Refer to the *Fluidics Station User's Guide* for instructions on connecting and addressing multiple fluidics stations.**

Priming the Fluidics Station

Priming ensures that the lines of the fluidics station are filled with the appropriate buffers and the fluidics station is ready for running fluidics station protocols.

Priming should be done:

- when the fluidics station is first started.
- when wash solutions are changed.
- before washing, if a shutdown has been performed.
- if the LCD window instructs the user to prime.

1. To prime the fluidics station, select **Protocol** in the Fluidics Station dialog box.
2. Choose **Prime** or **Prime_450** for the respective modules in the Protocol drop-down list.
3. Change the intake buffer reservoir A to **Non-Stringent Wash**

Buffer, and intake buffer reservoir B to Stringent Wash Buffer.

Probe Array Wash and Stain

After 16 hours of hybridization, remove the hybridization cocktail from the probe array and fill the probe array completely with the appropriate volume of Non-Stringent Wash Buffer (Wash Buffer A), as given in Table 5.1 on page 84.

NOTE **If necessary, at this point, the probe array can be stored at 4°C for up to 3 hours before proceeding with washing and staining. Equilibrate the probe array to room temperature before washing and staining.**

This protocol is recommended for use with probe arrays with probe cells of 24 µm or smaller. This procedure takes approximately 90 minutes to complete.The following tables list the various fluidics scripts and protocols to use depending on the arrays and reagents used in the assay.

Table 6.3
Fluidics Scripts for 11 µm Feature Size Eukaryotic Arrays*

	49	64	100	169	400
Using GeneChip® IVT Labeling Kit	EukGE-WS2v5	EukGE-WS2v5	Midi_euk2v3	Mini_euk2v3	Micro_1v1
Using all other labeling kits	EukGE-WS2v4	EukGE-WS2v4	Midi_euk2v3	Mini_euk2v3	Micro_1v1

** When using the Fluidics Station 450 or 250, add _450 at the end of the fluidics script's name.*

Table 6.4
Fluidics Scripts for ≥ 18 µm Feature Size Eukaryotic Arrays*

	49	64	100	169	400
Using GeneChip® IVT Labeling Kit	EukGE-WS2v4	EukGE-WS2v4	Midi_euk2v3	Mini_euk2v3	Micro_1v1
Using all other labeling kits	EukGE-WS2v4	EukGE-WS2v4	Midi_euk2v3	Mini_euk2v3	Micro_1v1

** When using the Fluidics Station 450 or 250, add _450 at the end of the fluidics script's name.*

Table 6.5
Fluidics Protocols - Antibody Amplification for Eukaryotic Targets
(protocols for the Fluidics Station 450/250 will have _450 as a suffix).

	EukGE-WS2v4*	EukGE-WS2v5* Midi_euk2*	Micro_1* Mini_euk2*
Post Hyb Wash #1	10 cycles of 2 mixes/cycle with Wash Buffer A at 25°C	10 cycles of 2 mixes/cycle with Wash Buffer A at 30°C	10 cycles of 2 mixes/cycle with Wash Buffer A at 25°C
Post Hyb Wash #2	4 cycles of 15 mixes/cycle with Wash Buffer B at 50°C	6 cycles of 15 mixes/cycle with Wash Buffer B at 50°C	8 cycles of 15 mixes/cycle with Wash Buffer B at 50°C
Stain	Stain the probe array for 10 minutes in SAPE solution at 25°C	Stain the probe array for 5 minutes in SAPE solution at 35°C	Stain the probe array for 10 minutes in SAPE solution at 25°C
Post Stain Wash	10 cycles of 4 mixes/cycle with Wash Buffer A at 25°C	10 cycles of 4 mixes/cycle with Wash Buffer A at 30°C	10 cycles of 4 mixes/cycle with Wash Buffer A at 30°C
2nd Stain	Stain the probe array for 10 minutes in antibody solution at 25°C	Stain the probe array for 5 minutes in antibody solution at 35°C	Stain the probe array for 10 minutes in antibody solution at 25°C
3rd Stain	Stain the probe array for 10 minutes in SAPE solution at 25°C	Stain the probe array for 5 minutes in SAPE solution at 35°C	Stain the probe array for 10 minutes in SAPE solution at 25°C
Final Wash	15 cycles of 4 mixes/cycle with Wash Buffer A at 30°C. The holding temperature is 25°C	15 cycles of 4 mixes/cycle with Wash Buffer A at 35°C. The holding temperature is 25°C	15 cycles of 4 mixes/cycle with Wash Buffer A at 35°C. The holding temperature is 25°C

- Wash Buffer A = non-stringent wash buffer
- Wash Buffer B = stringent wash buffer

** When using the Fluidics Station 450 or 250 add _450 at the end of the fluidics script's name.*

USING THE FLUIDICS STATION 450/250

Washing and Staining the Probe Array

1. In the Fluidics Station dialog box on the workstation, select the correct experiment name from the drop-down **Experiment** list.

   The **Probe Array Type** appears automatically.

2. In the **Protocol** drop-down list, select the appropriate antibody amplification protocol to control the washing and staining of the probe array format being used: Table 6.3 and Table 6.4.

3. Choose **Run** in the Fluidics Station dialog box to begin the washing and staining. Follow the instructions in the LCD window on the fluidics station.

   If you are unfamiliar with inserting and removing probe arrays from the fluidics station modules, please refer to the appropriate *Fluidics Station User's Guide* or *Quick Reference Card* (P/N 08-0093 for the FS-450/250 fluidics stations).

4. Insert the appropriate probe array into the designated module of the fluidics station while the cartridge lever is in the down, or eject, position. When finished, verify that the cartridge lever is returned to the up, or engaged, position.

5. Remove any microcentrifuge vial remaining in the sample holder of the fluidics station module(s) being used.

6. If prompted to "Load Vials 1-2-3," place the three experiment sample vials (the microcentrifuge vials) into the sample holders 1, 2, and 3 on the fluidics station.
   - Place one vial containing 600 µL of streptavidin phycoerythrin (SAPE) solution in sample holder 1.
   - Place one vial containing 600 µL of anti-streptavidin biotinylated antibody solution in sample holder 2.
   - Place one vial containing 600 µL of streptavidin phycoerythrin (SAPE) solution in sample holder 3.
   - Press down on the needle lever to snap needles into position and to start the run.

The run begins. The Fluidics Station dialog box at the workstation terminal and the LCD window display the status of the washing and staining as they progress.

7. At the end of the run, or at the appropriate prompt, remove the microcentrifuge vials and replace with three empty microcentrifuge vials.
8. Remove the probe arrays from the fluidics station modules by first pressing down the cartridge lever to the eject position.
9. Check the probe array window for large bubbles or air pockets.
   - If bubbles are present, proceed to Table 6.6.
   - If the probe array has no large bubbles, it is ready to scan on the GeneChip® Scanner 3000. Pull up on the cartridge lever to engage washblock and proceed to *Probe Array Scan* on page 104.

   If you do not scan the arrays right away, keep the probe arrays at 4°C and in the dark until ready for scanning.

   If there are no more samples to hybridize, shut down the fluidics station following the procedure outlined in the section, *Shutting Down the Fluidics Station* on page 107.

NOTE **For proper cleaning and maintenance of the fluidics station, including the bleach protocol, refer to Chapter 7, *Fluidics Station Care and Maintenance.***

For more information on the Fluidics Station 450/250, please refer to the *GeneChip® Fluidics Station 450 User's Guide.*

Table 6.6
If Bubbles are Present

Return the probe array to the probe array holder. Engage the washblock by gently pushing up on the cartridge lever to the engaged, or closed, position. The fluidics station will drain the probe array and then fill it with a fresh volume of the last wash buffer used. When it is finished, the LCD window will display **EJECT CARTRIDGE**. Again, remove the probe array and inspect it for bubbles. If no bubbles are present, it is ready to scan. Proceed to *Probe Array Scan* on page 104. If several attempts to fill the probe array without bubbles are unsuccessful, the array should be filled with **Wash Buffer A (non-stringent buffer)** manually, using a micropipette. Excessive washing will result in a loss of signal intensity.

USING THE FLUIDICS STATION 400

Washing and Staining the Probe Array

1. In the Fluidics Station dialog box on the workstation, select the correct experiment name in the drop-down **Experiment** list. The probe array type will appear automatically.
2. In the **Protocol** drop-down list, select the appropriate antibody amplification protocol to control the washing and staining of the probe array format being used: Table 6.3 and Table 6.4.
3. Choose **Run** in the Fluidics Station dialog box to begin the washing and staining. Follow the instructions on the LCD window on the fluidics station.
4. If you are unfamiliar with inserting and removing probe arrays from the fluidics station modules, please refer to the *Fluidics Station 400 User's Guide*, *Fluidics Station 400 Video In-Service CD* (P/N 900374), or *Quick Reference Card* (P/N 08-0072).
5. Insert the appropriate probe array into the designated module of the fluidics station while the cartridge lever is in the **EJECT** position. When finished, verify that the cartridge lever is returned to the **ENGAGE** position.
6. Remove any microcentrifuge vials remaining in the sample holder of the fluidics station module(s) being used.
7. When the LCD window indicates, place the microcentrifuge vial containing 600 µL of streptavidin phycoerythrin (SAPE) stain solution into the sample holder. Verify that the metal sampling needle is in the vial with its tip near the bottom.
8. When the LCD window indicates, replace the microcentrifuge vial containing the streptavidin phycoerythrin (SAPE) stain solution with a microcentrifuge vial containing antibody stain solution into the sample holder, making sure that the metal sampling needle is in the vial with its tip near the bottom.
9. When the LCD window indicates, replace the microcentrifuge vial containing the antibody stain solution with a microcentrifuge vial containing 600 µL of streptavidin phycoerythrin (SAPE) stain

solution into the sample holder. Verify that the metal sampling needle is in the vial with its tip near the bottom.

The Fluidics Station dialog box and the LCD window display the status of the washing and staining as they progress. When the wash is complete, the LCD window displays the message **EJECT CARTRIDGE.**

10. At the end of the run, or at the appropriate prompt, remove microcentrifuge vial containing stain and replace with an empty microcentrifuge vial.
11. Remove the probe arrays from the fluidics station modules by first moving the probe array holder lever to the **EJECT** position.
12. Check the probe array window for large bubbles or air pockets.
    - If bubbles are present, proceed to Table 6.7.
    - If the probe array has no large bubbles, it is ready to scan on the GeneChip® Scanner 3000. ENGAGE wash block and proceed to *Probe Array Scan* on page 104.

    If you do not scan the arrays right away, keep the probe arrays at 4°C and in the dark until ready for scanning.

    If there are no more samples to hybridize, shut down the fluidics station following the procedure outlined in the section, *Shutting Down the Fluidics Station* on page 107.

NOTE **For proper cleaning and maintenance of the fluidics station including the bleach protocol, refer to Chapter 7, *Fluidics Station Care and Maintenance*.**

Table 6.7
If Bubbles are Present

Return the probe array to the probe array holder. Latch the probe array holder by gently pushing it up until a light click is heard. Engage the wash block by firmly pushing up on the cartridge lever to the **ENGAGE** position. The fluidics station will drain the probe array and then fill it with a fresh volume of the last wash buffer used. When it is finished, the LCD window displays **EJECT CARTRIDGE** again, remove the probe array and inspect it again for bubbles. If no bubbles are present, it is ready to scan. Proceed to *Probe Array Scan* on page 104. If several attempts to fill the probe array without bubbles are unsuccessful, the array should be filled with **Wash Buffer A (non-stringent buffer)** manually, using a micropipette. Excessive washing will result in a loss of signal intensity.

Probe Array Scan

The GeneChip® Scanner 3000 is controlled by GCOS. The probe array is scanned after the wash protocols are complete. Make sure the laser is warmed up prior to scanning by turning it on at least 10 minutes before use. If probe array was stored at 4°C, warm to room temperature before scanning. Refer to the GCOS online help and the scanner user's manual for more information on scanning.

WARNING ▲ **The scanner uses a laser and is equipped with a safety interlock system. Defeating the interlock system may result in exposure to hazardous laser light.**

NOTE **You must have read, and be familiar with, the operation of the scanner before attempting to scan a probe array. Please refer to the GeneChip® Scanner 3000 Quick Reference Card (P/N 08-0075).**

HANDLING THE GENECHIP® PROBE ARRAY

Before you scan the probe array, follow the directions in this section on handling the probe array. If necessary, clean the glass surface of the probe array with a non-abrasive towel or tissue before scanning. Do not use alcohol to clean glass.

Before scanning the probe array cartridge, apply Tough-Spots® to each of the two septa on the probe array cartridge to prevent the leaking of fluids from the cartridge during scanning.

IMPORTANT ! **Apply the Tough-Spots just before scanning. Do not use them in the hybridization process.**

1. On the back of the probe array cartridge, clean excess fluid from around septa.
2. Carefully apply one Tough-Spots to each of the two septa. Press to ensure that the spots remain flat. If the Tough-Spots do not apply

smoothly, that is, if you observe bumps, bubbles, tears, or curled edges, do not attempt to smooth out the spot. Remove the spot and apply a new spot. See Figure 6.1.

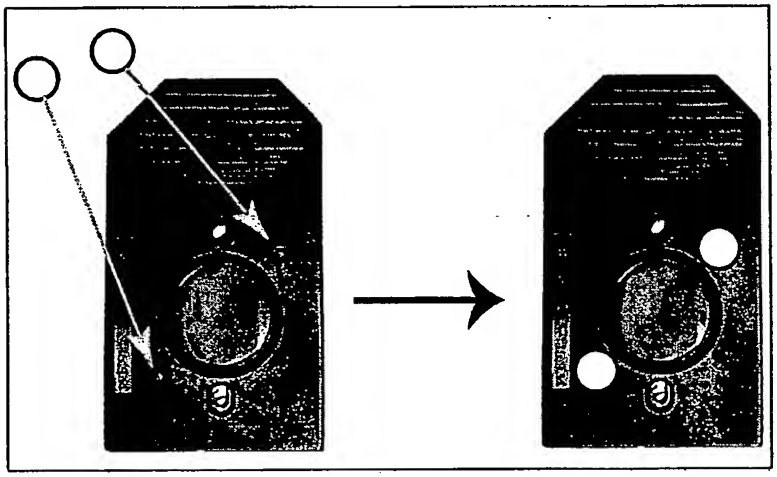


Figure 6.1
Applying Tough-Spots® to the probe array cartridge

3. Insert the cartridge into the scanner and test the autofocus to ensure that the Tough-Spots do not interfere with the focus. If you observe a focus error message, remove the spot and apply a new spot. Ensure that the spots lie flat.

SCANNING THE PROBE ARRAY

1. Select **Run** → **Scanner** from the menu bar. Alternatively, click the **Start Scan** icon in the tool bar.

   The Scanner dialog box appears with a drop-down list of experiments that have not been run.

2. Select the experiment name that corresponds to the probe array to be scanned.

   A previously run experiment can also be selected by using the **Include Scanned Experiments** option box. After selecting this option, previously scanned experiments appear in the drop-down list.

3. Once the experiment has been selected, click the **Start** button.

   A dialog box prompts you to load an array into the scanner.

4. Open the sample door on the scanner and insert the probe array into the holder. Do not force the probe array into the holder. Close the sample door of the scanner.

5. Click **OK** in the Start Scanner dialog box.

   The scanner begins scanning the probe array and acquiring data. When Scan in Progress is selected from the **View** menu, the probe array image appears on the screen as the scan progresses.

NOTE **For more information on the GeneChip® Scanner 3000 or the AutoLoader, please refer to the *GeneChip® Operating Software User's Guide* (P/N 701439).**

Shutting Down the Fluidics Station

1. After removing a probe array from the probe array holder, the LCD window displays the message ENGAGE WASHBLOCK.
2. If you are using the FS-400, latch the probe array holder by gently pushing up until a light click is heard. Engage the washblock by firmly pushing up on the cartridge lever to the ENGAGE position.

   If you are using the FS-450, gently lift up the cartridge lever to engage, or close, the washblock.

   The fluidics station automatically performs a Cleanout procedure. The LCD window indicates the progress of the Cleanout procedure.
3. When the fluidics station LCD window indicates REMOVE VIALS, the Cleanout procedure is complete.
4. Remove the sample microcentrifuge vial(s) from the sample holder(s).
5. If no other hybridizations are to be performed, place wash lines into a bottle filled with deionized water.
6. Choose **Shutdown** or **Shutdown_450** for all modules from the drop-down **Protocol** list in the Fluidics Station dialog box. Click the **Run** button for all modules.

   The Shutdown protocol is critical to instrument reliability. Refer to the appropriate *Fluidics Station User's Guide* for more information.
7. After Shutdown protocol is complete, flip the ON/OFF switch of the fluidics station to the OFF position.

IMPORTANT !

To maintain the cleanliness of the fluidics station and obtain the highest quality image and data possible, a weekly bleach protocol is highly recommended. Please refer to Chapter 7, *Fluidics Station Care and Maintenance* for further detail.

Customizing the Protocol

There may be times when the fluidics protocols need to be modified. Modification of protocols must be done before downloading the protocol to the fluidics station. Protocol changes will not affect runs in progress. For more specific instructions, refer to the GCOS online help.

1. Select Tools → **Edit Protocol** from the menu bar.

   The Edit Protocol dialog box appears.

2. Select the protocol to be changed from the **Protocol Name** drop-down list.

   The name of the protocol is displayed in the Protocol Name box. The conditions for that protocol are displayed on the right side of the Edit Protocol dialog box.

3. Select the items to be changed and input the new parameters as needed, keeping the parameters within the ranges shown below in Table 6.8.

Table 6.8
Valid Ranges for Wash/Stain Parameters

Parameter	Valid Range
Wash Temperature for A1, B, A2, or A3 (°C)	15 to 50
Number of Wash Cycles for A1, B, A2, or A3	0 to 99
Mixes / Wash cycle for A1, B, A2, or A3	1 to 99
Stain Time (seconds)	0 to 86399
Stain Temperature (°C)	15 to 50
Holding Temperature (°C)	15 to 50

- Wash A1 corresponds to Post Hyb wash #1 in Table Table 6.5.
- Wash B corresponds to Post Hyb wash #2 in Table Table 6.5.
- Wash A2 corresponds to Post Stain Wash in Table Table 6.5.
- Wash A3 corresponds to Final Wash in Table Table 6.5.

4. To return to the default values for the protocol selected, click the **Defaults** button.
5. After all the protocol conditions are modified as desired, change the name of the edited protocol in the **Protocol Name** box.

CAUTION **If the protocol is saved without entering a new "Protocol Name," the original protocol parameters will be overwritten.**

6. Click Save, then close the dialog box.

   Enter 0 (zero) for hybridization time if hybridization step is not required. Likewise, enter 0 (zero) for the stain time if staining is not required. Enter 0 (zero) for the number of wash cycles if a wash solution is not required.

Chapter 7

Fluidics Station Care and Maintenance

Chapter 7

Introduction

This chapter provides instructions on caring for and maintaining the instrument, and on troubleshooting if problems arise.

INSTRUMENT CARE

- Use a surge protector on the power line to the fluidics station.
- Always run a Shutdown protocol when the instrument will be off or unused overnight or longer. This will prevent salt crystals from forming within the fluidics system.
- When not using the instrument, leave the sample needles in the lowered position. Each needle should extend into an empty vial. This will protect them from accidental damage.
- Always use deionized water to prevent contamination of the lines. Change buffers with freshly prepared buffer at each system startup.
- The fluidics station should be positioned on a sturdy, level bench away from extremes in temperature and away from moving air.

IMPORTANT !

Before performing maintenance, turn off power to the station to avoid injury in case of a pump or electrical malfunction.

INSTRUMENT MAINTENANCE

To ensure proper functioning of the fluidics station, you should perform periodic maintenance.

Fluidics Station Bleach Protocol

Affymetrix recommends a weekly cleaning protocol for the fluidics station. This protocol uses commonly purchased sodium hypochlorite bleach.

This protocol is designed to eliminate any residual SAPE-antibody complex that may be present in the fluidics station tubing and needles. The protocol runs a bleach solution through the system followed by a rinse cycle with deionized (DI) water. This protocol takes approximately one hour and forty minutes to complete. Affymetrix recommends running this protocol weekly. You can find the current version of the protocol at:
www.affymetrix.com/support/technical/fluidics_scripts.affx.

THE BLEACH CYCLE

To avoid carryover, or cross contamination, from the bleach protocol, Affymetrix recommends the use of dedicated bottles for bleach and DI water. You can obtain additional bottles from Affymetrix.

Table 7.1
Affymetrix Recommended Bottles

Part Number	Description
400118	Media Bottle, SQ, 500 mL
400119	Media Bottle, SQ, 1000 mL

1. Disengage the washblock for each module by pressing down on the cartridge lever. Remove any probe array cartridge (Figure 7.1).

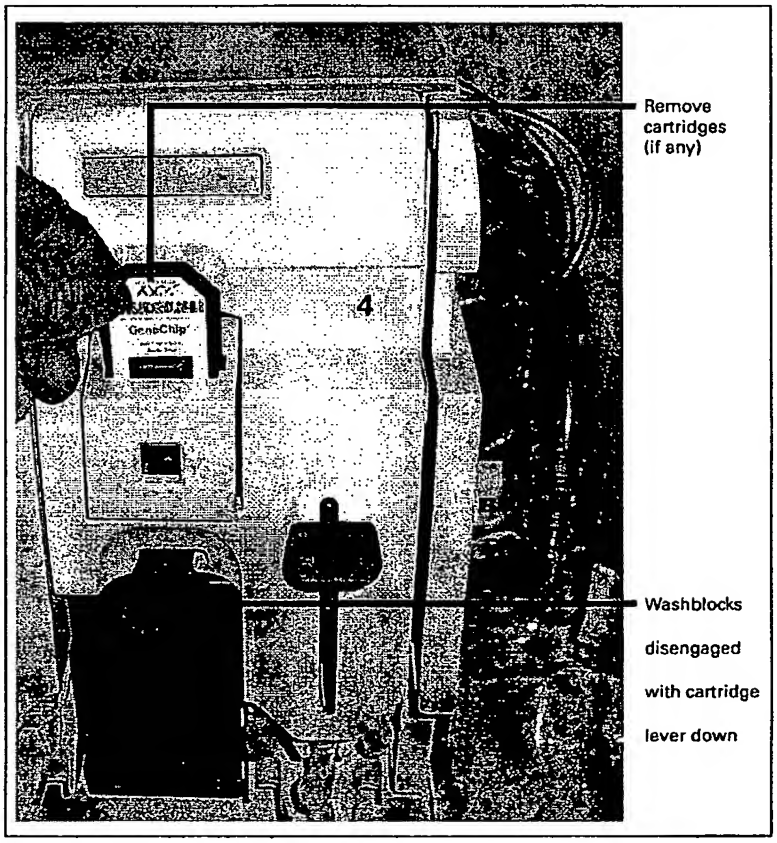


Figure 7.1
Disengaged washblocks showing cartridge levers in the down position, remove any cartridges

2. Prepare 500 mL of 0.525% sodium hypochlorite solution using deionized water. For example: follow these directions to make 500 mL of bleach.

   In a 1 liter plastic or glass graduated cylinder combine 43.75 mL of commercial bleach (such as Clorox bleach, which

is 6% sodium hypochlorite) with 456.25 mL of DI H_2O, mix well. Pour the solution into a 500 mL plastic bottle, and place the plastic bottle on fluidics station.

IMPORTANT **The shelf life of this solution is 24 hours. After this period, you must prepare a fresh solution.**

NOTE **Each fluidics station with four modules requires 500 mL of the 0.525% sodium hypochlorite solution.**

3. Place on the fluidics station an empty one liter waste bottle, a 500 mL bottle of bleach and a one liter bottle of DI water as shown in Figure 7.2. Insert the waste line into the waste bottle (Figure 7.2).
4. Immerse all three wash and water lines of the fluidics station into the 500 mL of bleach solution (Figure 7.2). DO NOT IMMERSE THE WASTE LINE INTO THE BLEACH.

NOTE **The BLEACH protocol requires approximately one liter of DI water.**

Figure 7.2
The bleach cycle. Immerse the tubes into the 0.525% sodium hypochlorite solution. The waste line remains in the waste bottle.

5. Open GeneChip Operating Software (GCOS).
   Click **Run** → **Fluidics...** from the menu. Alternatively, click the down arrow Protocol list on the toolbar. The protocol window appears (Figure 7.3).

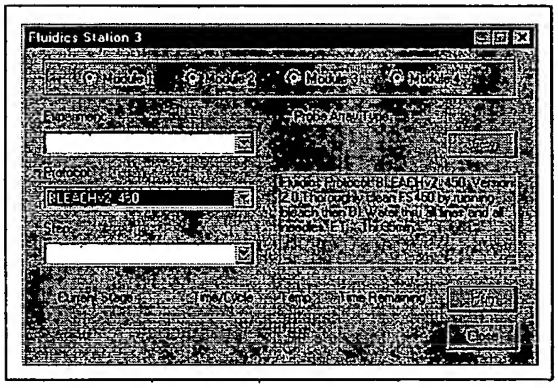


Figure 7.3
The Fluidics Station protocol window: select all modules.

6. Choose the current bleach protocol (in the current example, it is BLEACHv2_450) for each of the respective modules in the Protocol drop-down list. Select all four modules, 1 to 4, and click **Run**. The fluidics station will not start the bleach protocol until you press down on the needle lever (Figure 7.4).

NOTE **Temperature will ramp up to 50°C.**

7. Follow the prompts on each of the LCD. Load empty 1.5 mL vials onto each module if you have not already done so.
8. Press down on each of the needle levers to start the bleach protocol (Figure 7.4).

Figure 7.4
Press down on the needle levers to start the bleach protocol.

9. The fluidics station will begin the protocol and begin to empty the lines and perform the cleaning cycles using bleach solution.
10. After approximately 30 minutes, the LCD will prompt you when the bleach cycle is over and the rinse cycle about to begin.

THE RINSE CYCLE

Once the bleach cycle has finished, the second part of the protocol is a rinse step. This step is essential to remove all traces of bleach from the system. Failure to complete this step can result in damaged arrays.

1. Follow the prompts on the LCD for each module. Lift up on the needle levers and remove the bleach vials. Load clean, empty vials onto each module.
2. Remove the three wash and water lines from the bleach bottle and transfer them to the DI water bottle (Figure 7.5). At this step, you need not be concerned regarding the bleach that remains in the lines.

Figure 7.5
Immerse the three wash and water lines in the DI water bottle.

3. Press down on the needle levers to begin the rinse cycle. The fluidics station will empty the lines and rinse the needles.
4. When the rinse is completed after approximately one hour, the fluidics station will bring the temperature back to 25°C and drain the lines with air. The LCD display will read CLEANING DONE.
5. Discard the vials employed for the bleach protocol.
6. Follow these suggestions after you have completed the bleach protocol (Table 7.2).

Table 7.2
Quick Reference Guide to Using the FS-450

If you are:	Then do this:
Planning to use the system immediately	After running the bleach protocol, remove the DI water supply used in the rinse phase and install the appropriate reagents for use in your next staining and washing protocol (including fresh DI water). • Perform a prime protocol without loading your probe arrays. **Failure to run a prime protocol will result in irreparable damage to the loaded hybridized probe arrays.**
Not planning to use the system immediately	Since the system is already well purged with water, you need not run an additional shutdown protocol. Just remove the old DI water bottle and replace it with a fresh bottle.
Not planning to use the system for an extended period of time (longer than one week)	Remove the DI water and perform a "dry" protocol shutdown. This will remove most of the water from the system and prevent unwanted microbial growth in the supply lines. Also, remove the pump tubing from the peristaltic pump rollers.

NOTE **After you have completed the bleach protocol, discard the vials.**

Peristaltic Tubing Replacement

Periodically the peristaltic tubing requires replacement because of wear, contamination, or in order to avoid salt buildup. Inspect the tubing, if you see evidence of these conditions, follow the procedure outlined below.

IMPORTANT !

For systems in routine use, Affymetrix recommends monthly replacement of the tubing. To ensure proper performance, use only tubing available from Affymetrix. This tubing is manufactured to the required specifications to ensure proper fluid delivery and array performance. You can obtain additional tubing by ordering from Affymetrix:

Part Number	Description	Quantity
400110	Tubing, Silicone Peristaltic, 8.5	1

Wear gloves when changing tubing. Do not allow fluid from old tubing to spill onto surfaces.

1. Open the module door (Figure 7.6).

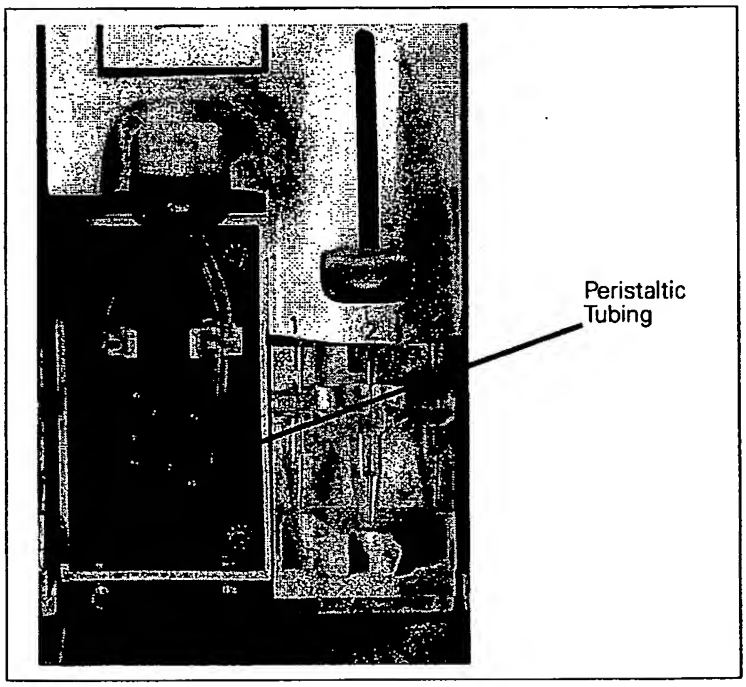


Figure 7.6
Module door open showing peristaltic tubing

2. Open the white clamps to release tubing on both sides. See Figure 7.7.

WARNING ▲ **Do not attempt to replace the tubing on a module where the module has been removed from the case of the fluidics station. In this case, rotating the pump may damage the motor driver circuitry.**

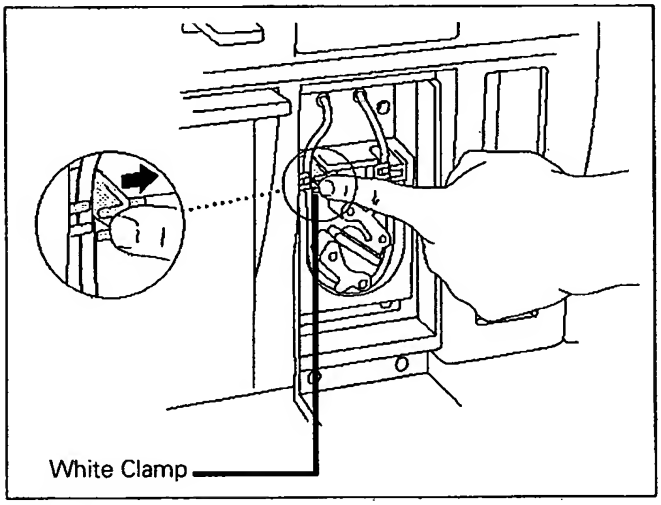


Figure 7.7
Releasing peristaltic tubing

3. Pull tubing off while gently turning the peristaltic pump head. Discard old tubing.
4. Replace tubing with new peristaltic tubing supplied with the accessory kit as described below:
   - **A.** Attach one end of the new tubing to the fitting on the right at the top of the pump enclosure.
   - **B.** Insert the tubing into the clamp under the fitting without stretching the portion of the tubing between the fitting and the clamp. There should be a small amount of slack in that portion of the tubing.
   - **C.** Work the tubing into the pump head while slowly turning the pump.
   - **D.** Insert the free end of the tubing into the other clamp, and attach it to the other fitting.
   - **E.** Close the drop-down module door.
5. Order more replacement tubing (P/N 400110).

Troubleshooting and Assistance

If problems arise with the fluidics station, use the following tables to locate the description that matches the problem. If you cannot find a solution, call Affymetrix Technical Support for assistance.

TROUBLESHOOTING DECISION TREE

The following simple flow charts (Figure 7.8 and Figure 7.9) show you how to begin troubleshooting the FS450/250 for a Missing Fluid Error (MFE).

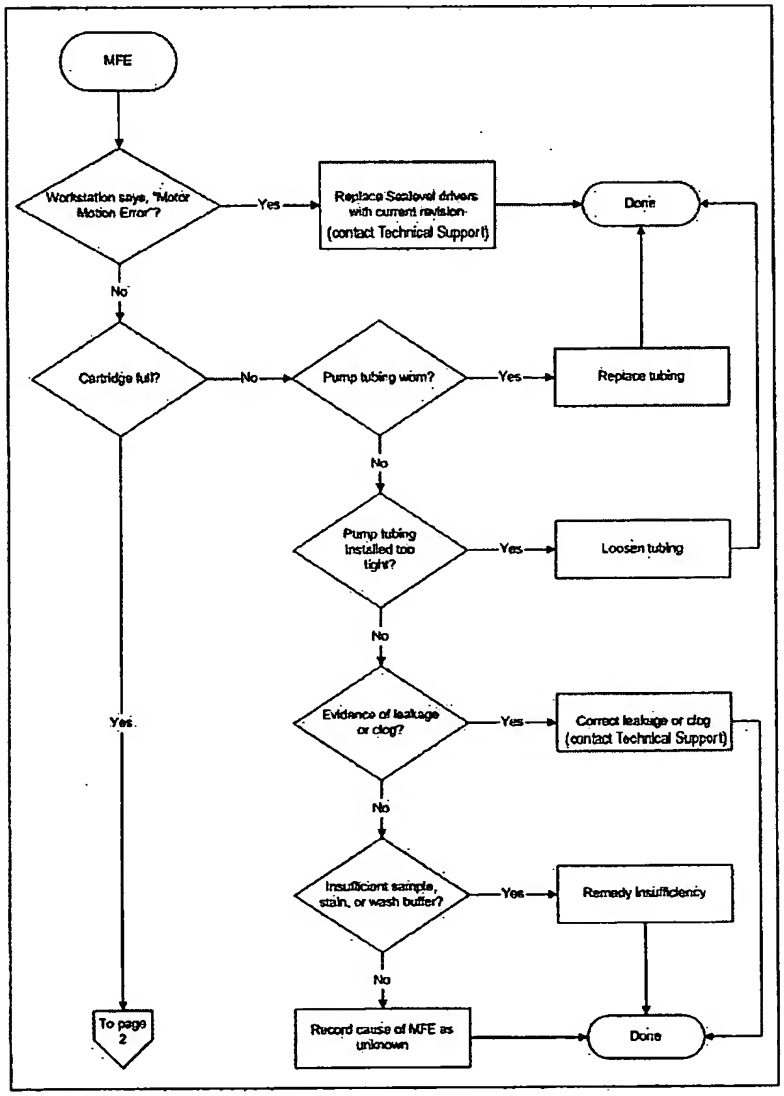


Figure 7.8
Troubleshooting decision tree, page 1

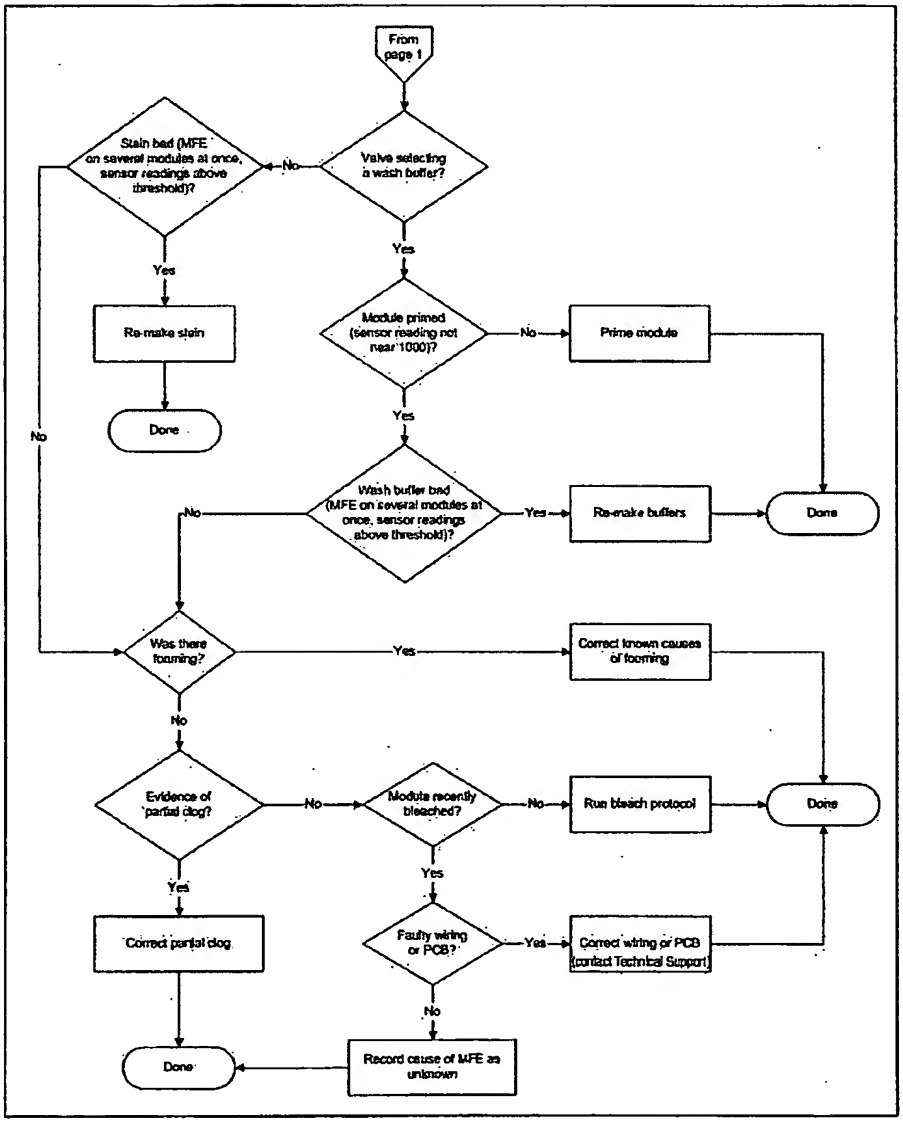


Figure 7.9
Troubleshooting decision tree, page 2

PROBLEMS AND SOLUTIONS

Table 7.3
Common error messages, their meanings, probable causes and solutions

Error Message	Problem	Possible Cause	Solution
Missing Fluid Error	Cartridge not filling completely with sample solution or buffer during initial stages of hybridization wash or staining protocol.	Possible holes in the septa of the cartridge.	Run Recover script, and then use another cartridge.
		Sample or staining solution not in place properly.	Run Recover script. Make sure sample or stain vial is in the sample holder.
		Insufficient volume of sample or staining solution (500 µL).	Run Recover script. Add more sample solution to the sample vial.
		Blocked sampling tube or line of the fluidics station.	Run Recover script. Run the Clean or Prime script with fresh deionized (DI) water to flush out salt blockage.
		Failure of one of the fluidics sensors.	Call Affymetrix Technical Support for service.
		Pump tubing stretched too tightly around the pump.	Loosen the tubing clamps, allow tubing to relax, close the clamps.
	Cartridge not filling completely with buffer during wash script	Buffer bottle empty.	Fill buffer bottles.
		Module not primed.	Prime module.
	System detects improper conditions while filling. Note where in protocol error occurred.	• Missing or insufficient stain or antibody in vial? • Wash empty? • Air bubbles in line? • Leaks?	Identify if chip is filled • If important to recover fluid in chip, and then run **Recovery** script, followed by **Resume** function • If not important to recover fluid in chip, run **Resume** function
	Recovered less sample than initial input during Recover script.	Loose tubing attachments inside the fluidics station.	Call Affymetrix Technical Support for service.

Table 7.3 (Continued)
Common error messages, their meanings, probable causes and solutions

Error Message	Problem	Possible Cause	Solution
Fluidics Station X Does Not Respond		Power not switched on at the fluidics station.	Turn fluidics station power on, and then try to connect again.
		Incorrect fluidics station designated for communication.	Designate correct fluidics station on workstation.
		Loose cables.	Firmly connect cables to fluidics station.
Sensor Timeout	"Sensor Timeout" error message on workstation.	No user response to "Remove Vial" prompt or other prompt.	Start the selected script again.
Error While Draining **Error While Filling**	Cartridge is not filling or draining properly.	Defective septa in cartridge.	Use a new cartridge.
		Insufficient sample or stain volume.	Add more sample solution to sample vial.
		Excessive bubbling in cartridge.	Change the buffer: reduce detergent.
		Buffer conductivity too low.	Change the buffer: increase salt.
		Failure of one of the fluid sensors.	Call Affymetrix Technical Support for service.
Error While Filling	System detects improper conditions while filling. Note where in protocol error occurred.	• Missing or insufficient stain or antibody in vial? • Wash or DI water empty? • Air bubbles in line? • Leaks?	Identify if chip is filled: • If important to recover fluid in chip, and then run **Recovery** script, followed by **Resume** function • If not important to recover fluid in chip, run **Resume** function

Table 7.3 (Continued)
Common error messages, their meanings, probable causes and solutions

Error Message	Problem	Possible Cause	Solution
Invalid Command	Communications error detected Note where in protocol error occurred		Identify if chip is filled. • If important to recover fluid in chip, and then run **Recovery** script. • Attempt to rerun script if sample loss can be tolerated. If problem persists, contact Affymetrix for service • If sample loss cannot be tolerated, do not attempt to rerun script. Contact Affymetrix for service
Temperature Timeout	Temperature does not reach specified temperature.	Temperature has not reached required level in expected time if ambient temperature is within operating specifications (15 – 30 degrees C).	Call Affymetrix Technical Support for service.
Improper Script	Script does not work.	User is attempting to run a FS400 script on FS450Dx	Download proper FS450Dx script and continue
Valve Motion Error			Run **Home** script and run desired script again If problem persists, contact Affymetrix for service.
Valve Not Homed			Run **Home** script and run desired script again If problem persists, contact Affymetrix for service.
Valve Out of Position			Run **Home** script and run desired script again If problem persists, contact Affymetrix for service.

MEANING OF ERROR MESSAGES

The following lists some of the common error messages and what they mean (Table 7.4).

Table 7.4
Common Error Messages

Error Message	Meaning
"Invalid Command"	The script contains a command that can not be executed because its command code is either undefined or has a format error.
"Improper Script"	The first command of the script is not the required FS450 command.
"Temperature Timeout"	The Re-attempt command timed out before the set point temperature was reached.
"Sensor Timeout"	The Await Sensors command timed out before the anticipated sensor pattern was seen.
"Valve not Homed"	The Home command did not result in the valve reaching it HOME position.
"Valve Motion Error"	The Valve command did not result in the valve reaching it target valve position.
"Valve out of Position"	According to the outer valve encoder, the valve did not reach a valid position when it was last rotated.
"Error while Filling"	While filling the cartridge, the AwaitMotor command terminated because of the step count not the expected sensor pattern, and that the same error had occurred several times consecutively.

Table 7.4 (Continued)
Common Error Messages

Error Message	Meaning
"Error while Draining"	While draining the cartridge, the AwaitMotor command terminated because of the step count not the expected sensor pattern, and that the same error had occurred several times consecutively.
"Missing Fluid Error" **Examples:** **"Stage C" "WashA"** **"Sense/Threshold" "960/890"**	"Stage C" "WashA" "Sense/Threshold" "960/890" The Pump command completed its step count before the conductivity sensor determined that the cartridge contained a solution with conductivity below the set threshold value. The Missing Fluid Error (MFE) Display not only gives a visual notification of an error condition to the operator, but gives the operator information that enables him/her to determine the cause of the error. It does this by displaying information about the sensor value and the fluid that caused the error. It shows this internal information in a continuous loop until the machine is powered down or a script is started. For example: Missing Fluid Error for 4 seconds Stage A valvePos WashA for 4 seconds Sense/Threshold 820/600 for 4 seconds

OTHER PROBLEMS AND SOLUTIONS

Table 7.5 lists other problems, causes and solutions that you may encounter.

Table 7.5
Other Problems

Problem	Possible Cause	Solution
Air bubbles left in cartridge at the end of a hybridization-wash script.	Air bubble in wash line	Manually fill cartridge with Buffer A.
Buffer leaking inside the fluidics station.	Loose tubing attachments inside the fluidics station.	Call Affymetrix Technical Support for service.
	Washblock requires replacement.	Call Affymetrix Technical Support for service.
	Salt buildup in the lines of the fluidics station.	Run the Clean or Prime script with fresh DI water to flush out salt blockage.
Cartridge needles of the fluidics station not engaging with the cartridge.	Possible defective septa on the cartridge.	Use another cartridge.
	Extra flashing on the cartridge.	Use another cartridge, or call Affymetrix Technical Support for service.
	Salt buildup on the cartridge needles.	Run the Clean script with fresh DI water to flush out salt blockage. Clean cartridge needles with a wet cotton swab.
	Cartridge holder aligned and attached to the fluidics station improperly.	Call Affymetrix Technical Support for service.
	Cartridge holder not properly engaged to the fluidics station.	Place the cartridge into the cartridge holder. Push the holder door shut, and firmly lift the lever to engage the cartridge needles.

Table 7.5 (Continued)
Other Problems

Problem	Possible Cause	Solution
Sample needles do not properly enter vial.	Bent sample needle	Replace sample needle.
	User may be pressing the needle lever down to quickly or with too much force.	Engage sample needle lever more slowly and/or with less force.

INSTRUMENT SPECIFICATIONS

Fluidics Station Dimensions:

(height, depth, width)
40.2 x 41.0 x 71.1 cm or 15 13/16 x 16 1/8 x 28 inches

Product Weight:

Approximately 80 pounds or 36.3 kg

Power Input:

100 to 240 V~, 3 A
300 watts or less. Main supply voltage fluctuations not to exceed 15% of the nominal supply voltage.

Temperature:

Operating: 15° to 30°C
Storage (non-operating):-10° to 60°C

Humidity:

Operating: 10-90% RH, non-condensing
Storage (non-operating):10% to 95% RH

Other:

Pollution degree, 2
Installation category, II

Electrical Supply

The electrical supply shall meet the input specified on the instrument label. Voltage fluctuations shall not exceed 15% nominal supply voltage.

Altitude

<2000 m

Appendix A

Master Mix Spreadsheet

Appendix A

Master Mix Volume Spreadsheets

The following pages contain spreadsheets of the master mix volumes for the different reaction configurations.

The GeneChip® Array Station is designed to facilitate higher-throughput target preparation for the GeneChip Expression Assay. Reagent volumes provided in the GeneChip® HT cDNA Synthesis Kit (P/N 900687) and the GeneChip® HT IVT Labeling Kit (P/N 900688) are sufficient for four 24-sample master mixes, providing overage to accommodate for pipetting error. Occasionally, smaller sample master mixes may be useful. Formulations for several master mix formulations, accommodating various sample sizes, are provided in the following tables. Please note in the tables that the "volume per strip tube" values for each reagent is adjusted to sufficiently accommodate the requirements of the GeneChip Array Station.

Table A.1
T7 Primer Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
T7-Oligo(dT) Primer, 50 µM	1.0	12.0	23.2	32.0	38.0	47.5	57.0	66.5	76.0	85.5	95.0	104.5	114.0
Nuclease-free Water	4.0	48.0	92.8	127.9	152.0	190.0	228.0	266.0	304.0	342.0	380.0	418.0	456.0
Total Volume	5.0	60.0	116.0	159.9	190.0	237.5	285.0	332.5	380.0	427.5	475.0	522.5	570.0
Vol per Strip Tube Well		7.0	13.5	18.5	22.0	28.0	34.0	40.0	45.5	51.4	57.4	63.3	69.3

Table A.2
First-Strand cDNA Synthesis Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
5X 1st Strand Rxn Mix	4.0	46.4	82.8	114.0	152.0	190.0	228.0	266.0	304.0	342.0	380.0	418.0	456.0
DTT, 0.1 M	2.0	23.2	41.4	57.0	76.0	95.0	114.0	133.0	152.0	171.0	190.0	209.0	228.0
dNTP Mix, 10 mM	1.0	11.6	20.7	28.5	38.0	47.5	57.0	66.5	76.0	85.5	95.0	104.5	114.0
SuperScript™ II	1.0	11.6	20.7	28.5	38.0	47.5	57.0	66.5	76.0	85.5	95.0	104.5	114.0
Nuclease-free Water	2.0	23.2	41.4	57.0	76.0	95.0	114.0	133.0	152.0	171.0	190.0	209.0	228.0
Total Volume	10.0	116.0	207.0	285.0	380.0	475.0	570.0	665.0	760.0	855.0	950.0	1,045.0	1,140.0
Vol per Strip Tube Well		14.0	24.0	33.5	45.5	57.4	69.3	81.1	93.0	104.9	116.8	128.6	140.5

Table A.3
Second-Strand cDNA Synthesis Master Mix

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
5X 2nd Strand Rxn Mix	30.0	270.0	540.0	810.0	1,080.0	1,350.0	1,620.0	1,890.0	2,160.0	2,430.0	2,700.0	2,970.0	3,240.0
dNTP Mix, 10 mM	3.0	27.0	54.0	81.0	108.0	135.0	162.0	189.0	216.0	243.0	270.0	297.0	324.0
DNA Ligase, 10 unit/µL	1.0	9.0	18.0	27.0	36.0	45.0	54.0	63.0	72.0	81.0	90.0	99.0	108.0
DNA Polymerase I, 10 unit/µL	4.0	36.0	72.0	108.0	144.0	180.0	216.0	252.0	288.0	324.0	360.0	396.0	432.0
RNase H, 2 unit/µL	1.0	9.0	18.0	27.0	36.0	45.0	54.0	63.0	72.0	81.0	90.0	99.0	108.0
Total Volume	39.0	351.0	702.0	1,053.0	1,404.0	1,755.0	2,106.0	2,457.0	2,808.0	3159.0	3,510.0	3,861.0	4,212.0
Vol per Strip Tube Well		41.0	85.0	129.0	172.0	216.0	260.0	303.0	347.0	391.0	435.0	479.0	522.5

Table A.4
T4 DNA Polymerase Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
T4 DNA Polymerase	2.0	30.7	49.7	69.3	81.1	98.3	118.0	137.7	157.3	177.0	196.7	216.3	236.0
1X T4 DNA Polymerase Buffer	2.0	30.7	49.7	69.3	81.1	98.3	118.0	137.7	157.3	177.0	196.7	216.3	236.0
Total Volume	4.0	61.4	99.4	138.6	162.2	196.6	236.0	275.4	314.6	354.0	393.4	432.6	472.0
Vol per Strip Tube Well		7.0	11.0	16.0	18.5	23.0	27.8	33.0	38.0	42.3	47.3	52.1	57.0

Table A.5
IVT Cocktail for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
10X IVT Buffer	6.0	56.0	112.0	168.0	224.0	280.0	336.0	392.0	448.0	504.0	560.0	616.0	672.0
IVT Labeling NTP Mix	18.0	168.0	336.0	504.0	672.0	840.0	1,008.0	1,176.0	1,344.0	1,512.0	1,680.0	1,848.0	2,016.0
IVT Labeling Enzyme Mix	6.0	56.0	112.0	168.0	224.0	280.0	336.0	392.0	448.0	504.0	560.0	616.0	672.0
T7 RNA Polymerase	1.0	9.3	18.7	28.0	37.3	46.7	56.0	65.3	74.7	84.0	93.3	102.7	112.0
Nuclease-free Water	7.0	65.3	130.7	196.0	261.3	326.7	392.0	457.3	522.7	588.0	653.3	718.7	784.0
Total Volume	38.0	354.6	709.4	1,064.0	1,418.6	1,773.4	2,128.0	2,482.6	2,837.4	3,192.0	3,546.6	3,901.4	4,256.0
Vol per Strip Tube Well		43.0	87.0	131.0	174.0	219.0	264.0	308.0	352.0	396.0	440.0	484.0	529.0

Table A.6
Fragmentation Cocktail for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
5X Fragmentation Buffer (Vol per Strip Tube Well)	7.5	11.4	17.8	26.7	35.6	44.5	53.4	62.3	71.3	80.2	89.1	98.0	106.9

Table A.7
Hybridization Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
3 nM B2 Oligo	4.95	44.6	89.1	133.7	178.2	222.8	267.3	311.9	356.4	401.0	445.5	490.1	534.6
20X *BioB, C, D, Cre* (control)	15.0	135.0	270.0	405.0	540.0	675.0	810.0	945.0	1,080.0	1,215.0	1,350.0	1,485.0	1,620.0
HS DNA (10 mg/mL)	3.0	27.0	54.0	81.0	108.0	135.0	162.0	189.0	216.0	243.0	270.0	297.0	324.0
BSA (50 mg/mL)	3.0	27.0	54.0	81.0	108.0	135.0	162.0	189.0	216.0	243.0	270.0	297.0	324.0
2X Hyb Buffer	150.0	1,350.0	2,700.0	4,050.0	5,400.0	6,750.0	8,100.0	9,450.0	10,800.0	12,150.0	13,500.0	14,850.0	16,200.0
DMSO (100%)	30.0	270.0	540.0	810.0	1,080.0	1,350.0	1,620.0	1,890.0	2,160.0	2,430.0	2,700.0	2,970.0	3,240.0
Nuclease-free Water	64.05	576.5	1,152.9	1,729.4	2,305.8	2,882.3	3,458.7	4,035.2	4,611.6	5,188.1	5,764.5	6,341.0	6,917.4
Total Volume	270.0	2,430.1	4,860.0	7,290.1	9,720.0	12,150.1	14,580.0	17,010.1	19,440.0	21,870.1	24,300.0	26,730.1	29,160.0
# of QIAGEN Strips Used		1	1	2	2	3	3	4	4	5	5	6	6
Volume per Well 1st Strip		300	600	600	600	600	600	600	600	600	600	600	600
Volume per Well 2nd Strip				300	600	600	600	600	600	600	600	600	600
Volume per Well 3rd Strip						300	600	600	600	600	600	600	600
Volume per Well 4th Strip								300	600	600	600	600	600
Volume per Well 5th Strip										300	600	600	600
Volume per Well 6th Strip												300	600

Appendix B Automated Sample Transfer

Appendix B

Loading Sample Plate and Initial Deck Layout for Automated Sample Transfer

The Automated Sample Transfer function uses the Array Station to transfer 5 µL of total RNA from a Greiner U-bottom plate to a lidded Bio-Rad 96-Well Hard-Shell PCR Plate.

PROCEDURE

1. Set up the deck with the appropriate consumables, as illustrated in Figure 4.18 on page 56.
2. Select the protocol parameters to enable the **Continue Run** button (Figure B.1).

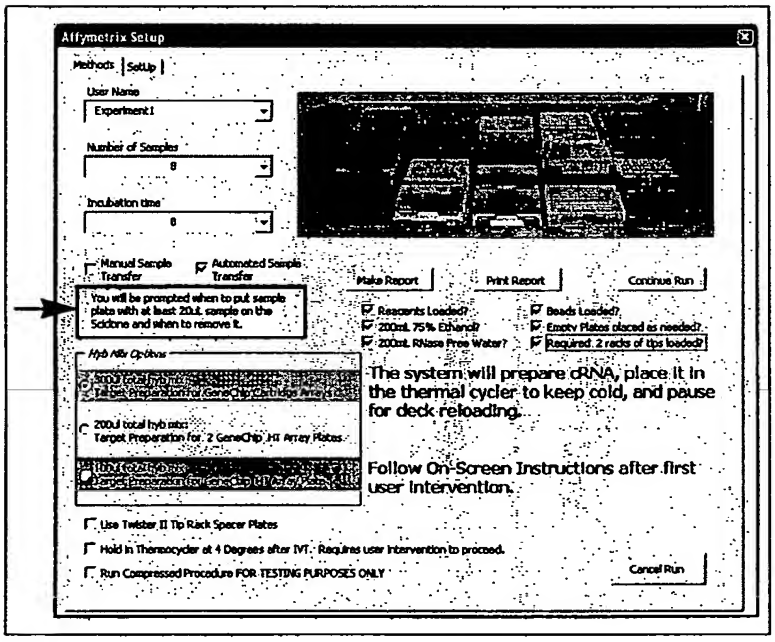


Figure B.1

A. From the drop-down option boxes, select User Name, Number of Samples by 8's (by column starting with position A1), and IVT Incubation Time.

B. Select the **Automated Sample Transfer** option.

C. To make the final hybridization cocktail for the cartridge arrays, select the radio button for the **300 µL total hyb mix.**

D. There are three option at the bottom of the **Methods** tab in the Application Setup Window:

1) Select the first option, "Use Twister II Tip Rack Spacer Plates" when using the spacer plates if there is a static electricity concern.

2) Select the second option, "Hold in Thermal Cycler at 4 Degrees after IVT. Requires user intervention to proceed." to enable post IVT procedures to start at a designated time.

3) The third option, "Run Compressed Procedure FOR TESTING PURPOSES ONLY," is for testing purposes only and should not be selected.

E. When appropriate options are selected, click the **Make Report** button. Immediately, the six check boxes under **Make Report** are enabled.

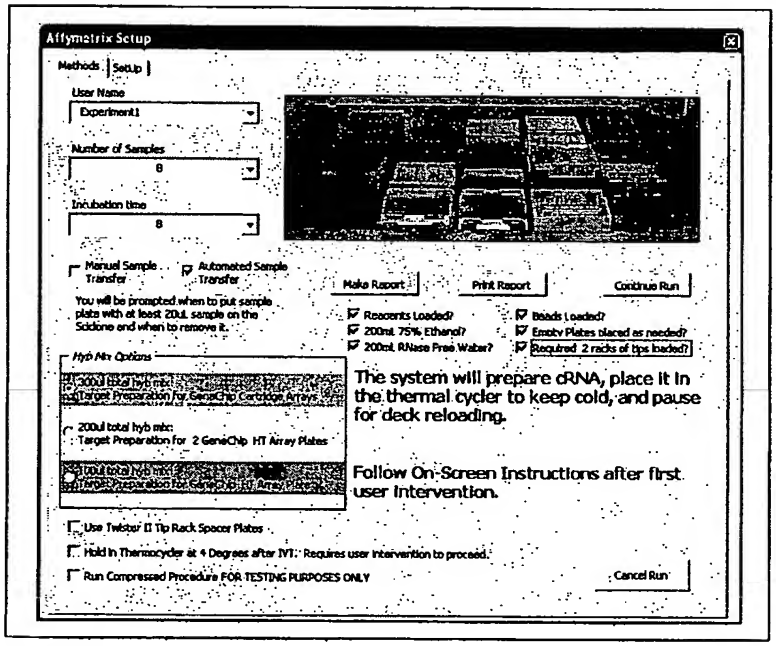


Figure B.2
Application Setup Window

NOTE

To cancel before continuing the run follow these steps:

1. **Click the "End Now" button in iLink.**
2. **Click the "Cancel Run" button.**
3. **Two windows appear in succession.**
4. **Click "Yes" and "OK" sequentially to cancel the run.**

F. Use the six-option checklist on the right side of the window to ensure all reagents and consumables are loaded, and select each option (all six boxes should be checked as in Figure B.2). The Continue Run button becomes enabled.

G. Click **Continue Run**.

3. After priming and initialization, the message, illustrated in Figure B.3, will prompt you to remove the Bio-Rad 96-Well Hard-Shell PCR Plate in D2 and replace it with the Greiner U-bottom sample plate with at least 20 µL of sample.

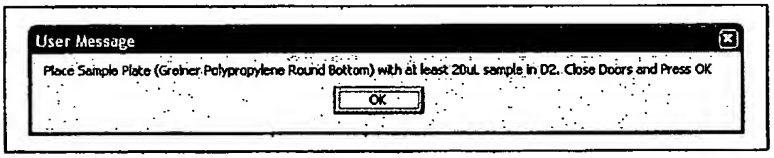


Figure B.3

4. After transfer is complete, Figure B.4 appears.

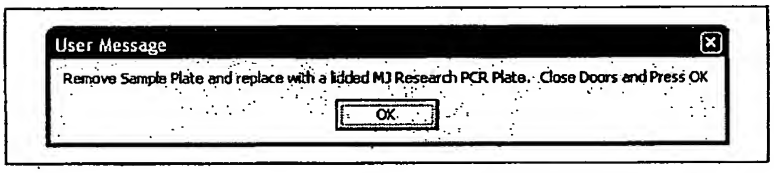


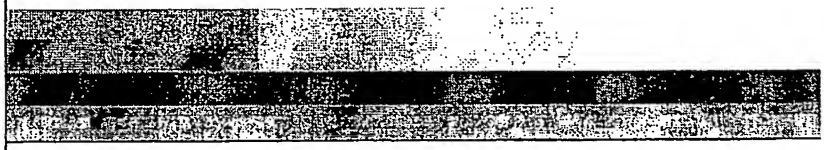
Figure B.4

5. Remove the Greiner plate from the D2 position and replace it with the "unfrag cRNA" plate 1. Close the doors to the Array Station and select OK to proceed.

Appendix C

Array Station Applications Spreadsheet

Appendix C



Spreadsheets Loaded for the Target Preparation Protocol

After selecting the **Continue Run** button during the Application Setup Window, iLink loads the Excel spreadsheets listed in this Appendix.

NOTE **Do not open, close, or alter spreadsheets during a run.**

1. The first two sheets show the deck layouts (Figure C.1 and Figure C.2).

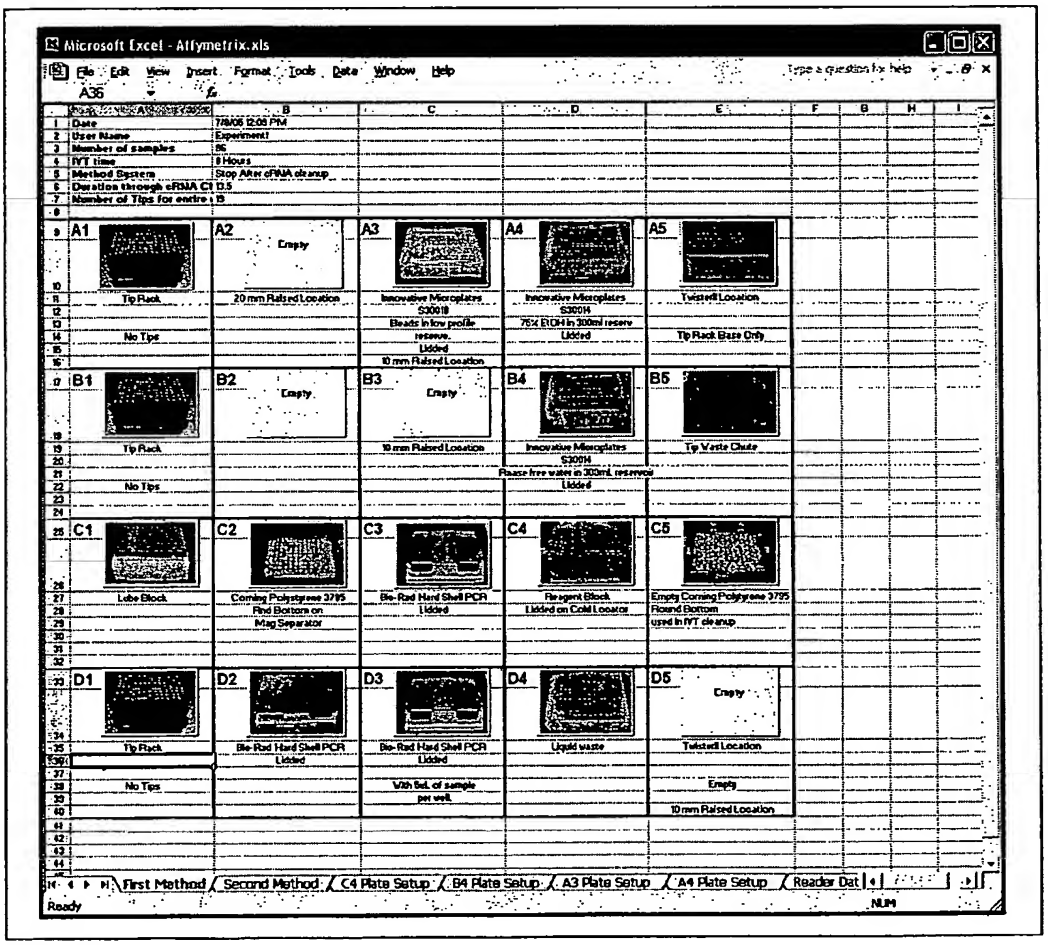


Figure C.1
1st Layout Spreadsheet

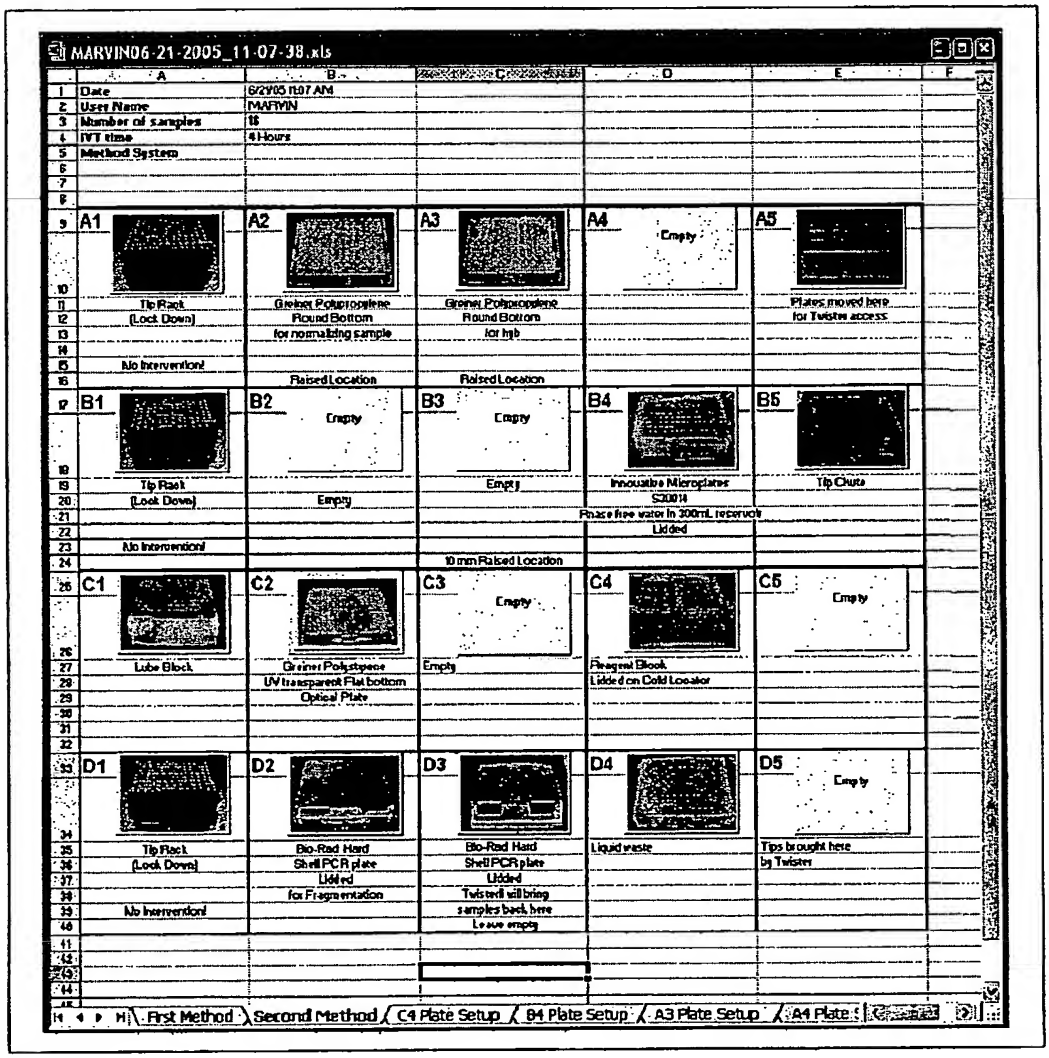


Figure C.2
2nd Layout Spreadsheet

2. The third sheet, C4 Plate Setup, has the cold reagent block setup with reagent amounts calculated for the number of samples run, as well as a record of selected options (Figure C.3).

NOTE **The volumes represented in the Excel spreadsheet (Figure C.3) may not match the volumes listed in this manual. The volumes listed in this manual are the most up-to-date and should be followed. Refer to the most recent version of this manual to obtain the most current values.**

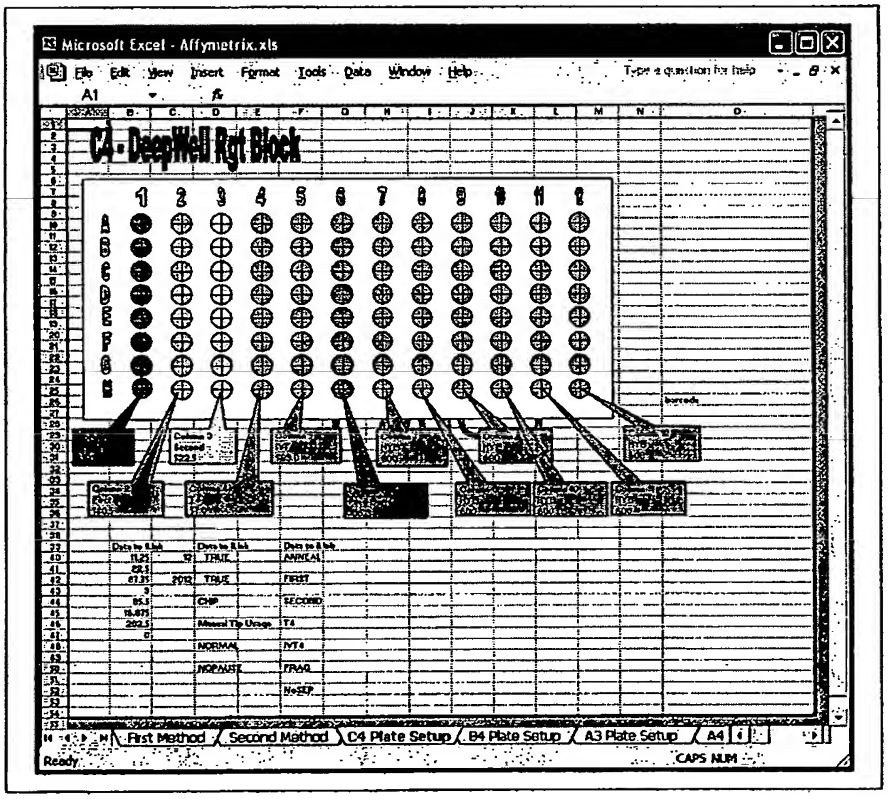


Figure C.3
C4 Plate Setup

3. The next three spreadsheets provide information on water, ethanol and bead reservoir fill volumes (Figure C.4).

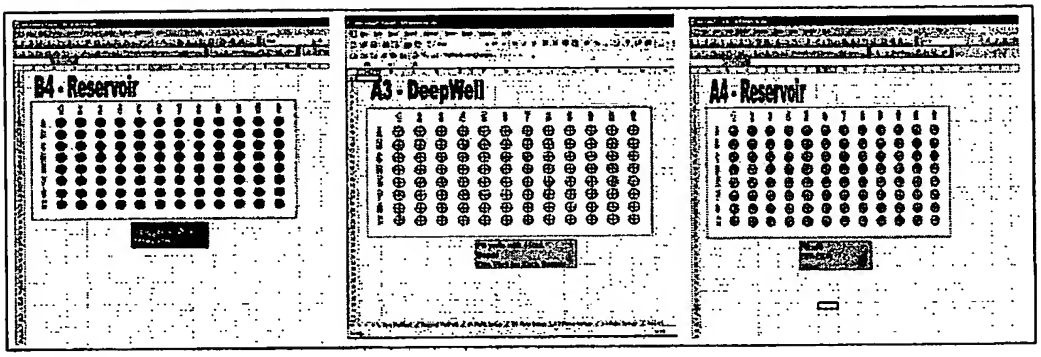


Figure C.4

4. The Setup and Version Number sheets are necessary for the initial user interface (Figure C.5).

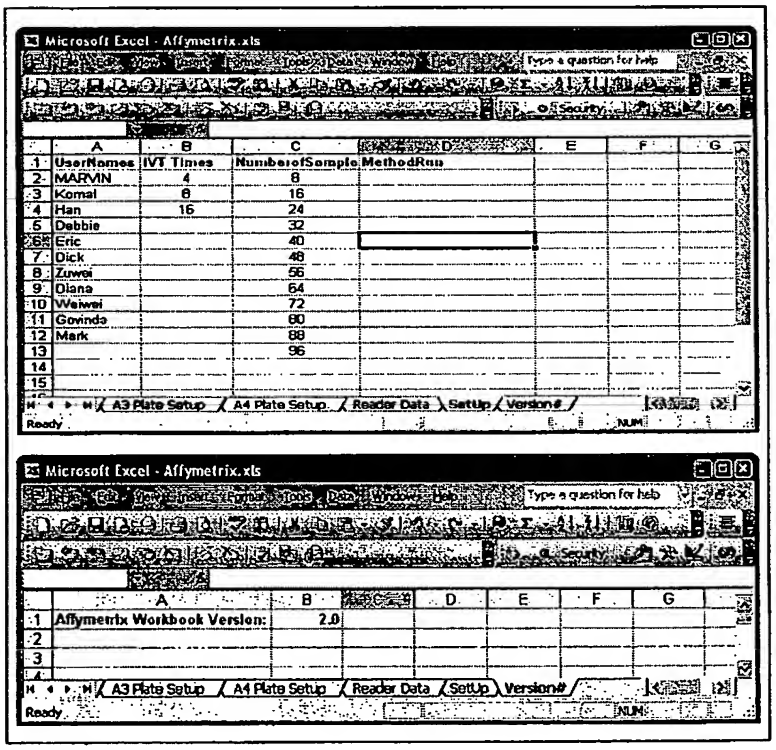


Figure C.5

5. The Reader Data sheet is automatically filled at the 1st and 2nd quantitation steps (Figure C.6).

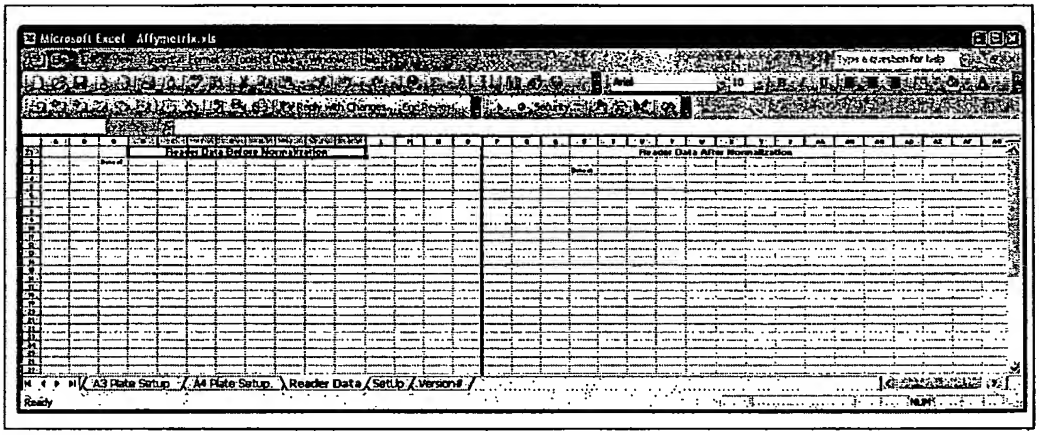


Figure C.6

6. The Tip Usage Excel workbook is used to determine tip loading parameters for each number of samples run as well as calculating sample yields and normalization volumes from sample ODs during the quantitation steps. This normalization data is copied to the Reader Data page of the first (Affymetrix.xls) workbook (Figure C.7).

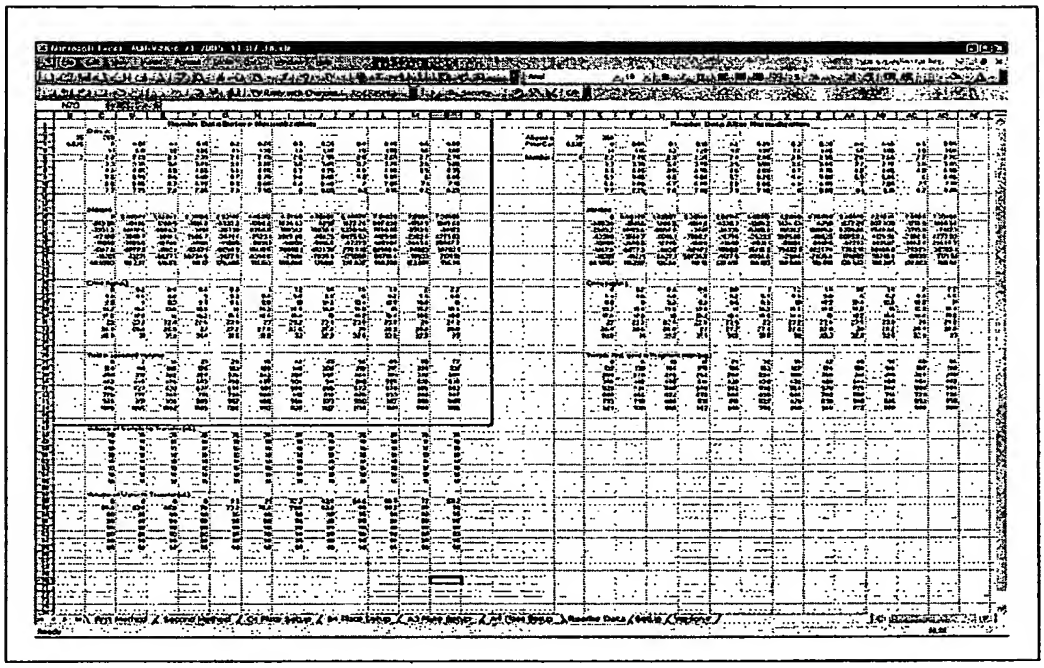


Figure C.7

Appendix D

Reagents, Equipment, and Supplier Contact Information

Appendix D

Master List - Consumables, Reagents, and Equipment

TARGET PREPARATION

You will need the following reagents and supplies to complete the target preparation on the Array Station system. The reagent quantities listed are for one plate of 96 well reactions.

IMPORTANT ! **All labware, including pipettes, must be RNase/DNase-free.**

INSTRUMENTS

Table D.1
Instruments

Instruments	Supplier	P/N	Quantity / Run
GeneChip® Array Station	Affymetrix		
GeneChip® Hybridization Oven 640	Affymetrix	800138 (110V) or 800139 (220V)	
GeneChip® Scanner 3000	Affymetrix	00-0074	
12-multichannel 200 µL Pippetman	Rainin	L12-200	
8-multichannel 200 µL Pippetman	Rainin	L8-200	
12-multichannel 20 µL Pippetman	Rainin	P/N L12-20	
8-multichannel 20 µL Pippetman	Rainin	P/N L8-20	
Multidispensing pipette - 200 µL	Rainin	P/N E3-200	
Multidispensing pipette - 1000 µL	Rainin	P/N E3-1000	
Zerostat Anti-Static Gun	Audioadvisor.com	Zerostat	
Heatblock	multiple		
Sealing Roller	Bio-Rad	MSR-0001	
Microseal P pads ADHESIVE	Bio-Rad	MSP-1002	1 (100 seals)
Auto-sealing microplate lid, arched, wide tab	Bio-Rad	MSL 2032	4
Gripper Pads	Caliper LifeSciences	52071	8

CONSUMABLES

Table D.2
Consumables

Item	Source	P/N	Quantity / Run
96-Well Hard-Shell PCR Plate	Bio-Rad	HSP-9601	4
Corning Polystyrene Round Bottom Plates	Fisher Scientific	CLS3795	2
Greiner UV transparent Optical Plates	E&K Scientific Products	EK-25801	2
1.2 mL Square Well Storage Plate, Low Profile	ABGene/Marsh	AB-1127	1
Elution Strip Tubes, 0.85 mL	QIAGEN	19588	8
96 Wells High Profile 300 mL Reservoir	E&K Scientific Products	EK-2035	2
Low-Profile 0.2 mL PCR 8-Tube Strips	Bio-Rad	TLS-0801	4
Microtiter plate lids	Phenix	ML-5009	3
Greiner round bottom clear polypropylene plate	E&K Scientific Products	20261	2
Stacker tips 200 µL non-sterile	Caliper LifeSciences	78641	25 racks
BD Falcon™ Test Tube, 5 mL	VWR International	60819-728	3
BD Falcon™ Test Tube, 14 mL	VWR International	60819-761	3
Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL	VWR International	20171-028	2
RNase-Free 1.5 mL Microfuge tube*	Ambion	12400	7
RNaseZap® wipes	Ambion	9786	1 (100 wipes)
DNAZap™	Ambion	9890	1 (2 bottles)
KimWipes®	VWR International	34256	
Microseal 'F' Adhesive Foil	Bio-Rad	MSF-1001	
Sterile-barrier, RNase-free pipette tips†	multiple		

**or equivalent*

†Tips must be pointed, not rounded, for efficient use with the probe arrays. Beveled tips may cause damage to the array septa and cause leakage.

REAGENTS

Table D.3
Reagents

Material	Source	P/N
Total RNA Isolation		
TRIzol® Reagent	Invitrogen Life Technologies	15596-018
QIAzol™ Lysis Reagent	QIAGEN	79306
RNeasy® Mini Kit	QIAGEN	74104
ethanol, 80% (stored at –20°C)		
Pellet Paint® (optional)	Novagen	69049-3
Glycogen (optional)	Ambion	9510
Sodium Acetate (NaOAc), 3 M	Sigma-Aldrich	S7899
Target Preparation		
GeneChip® HT One-Cycle Target Labeling and Controls Kit (30 samples) containing: • GeneChip® HT One-Cycle cDNA Synthesis Kit 96-Reactions, P/N 900687 • GeneChip® HT IVT Labeling Kit 96-Reactions, P/N 900688 • GeneChip® Eukaryotic Poly-A RNA Control Kit 100-Reactions, P/N 900433 • GeneChip® Eukaryotic Hybridization Control Kit 150-Reactions, P/N 900457	Affymetrix	900686
GeneChip® Eukaryotic Poly-A RNA Control Kit containing: • Poly-A Control Stock (16 µL) • Poly-A Control Dil Buffer (3.8 mL)	Affymetrix	900433

Table D.3 (Continued)
Reagents

Material	Source	P/N
GeneChip® HT One-Cycle cDNA Synthesis Kit containing: • T7-Oligo(dT) Primer, 50 µM (130 µL) • 5X 1st Strand Reaction Mix (460 µL) • DTT, 0.1 M (230 µL) • dNTP, 10 mM (460 µL) • SuperScript™ II (120 µL) • 5X 2nd Strand Reaction Mix (3,300 µL) • *E. coli* DNA Polymerase I (440 µL) • *E. coli* DNA Ligase (110 µL) • RNase H (110 µL) • T4 DNA Polymerase (280 µL) • 5X T4 DNA Polymerase Buffer (60 µL)	Affymetrix	900687
GeneChip® HT IVT Labeling Kit containing: • 10X IVT Labeling Buffer, 1 tube (675 µL) • IVT Labeling Enzyme Mix, 1 tube (675 µL) • IVT Labeling NTP Mix, 2 tubes (1,010 µL) • 3'-Labeling Control (0.5 µg/µL), 1 tube (10 µL) • T7 RNA Polymerase, 1 tube (115 µL) • 5X Fragmentation Buffer, 1 tube (855 µL)	Affymetrix	900688
Nuclease-free Water	Ambion	9932
RNAClean 60 mL	Agencourt	000494
Target Hybridization		
Nuclease-free Water	Ambion	9932
Bovine Serum Albumin (BSA) solution (50 mg/mL)	Invitrogen Life Technologies	15561-020
Herring Sperm DNA	Promega Corporation	D1811
GeneChip® Eukaryotic Hybridization Control Kit contains: • Control cRNA and Control Oligo B2	Affymetrix	900454 (30 Rxns) or 900457 (150 Rxns)
Control Oligo B2, 3 nM (can be ordered separately)	Affymetrix	900301
5 M NaCl, RNase-free, DNase-free,	Ambion	9760G

Table D.3 (Continued)
Reagents

Material	Source	P/N
MES hydrate SigmaUltra	Sigma-Aldrich	M5287
MES Sodium Salt	Sigma-Aldrich	M5057
EDTA Disodium Salt, 0.5 M solution (100 mL)	Sigma-Aldrich	E7889
DMSO	Sigma-Aldrich	D5879
Surfact-Amps® 20 (Tween-20), 10%	Pierce Chemical	28320
Nuclease-Free Water, 1 L	Ambion	9932
RNAClean™ 60 mL Kit	Agencourt	000494
Ethanol 100%, 1 L	Aldrich	45984-4
Washing, Staining, and Scanning		
DEPC-Treated Water	Ambion	9920
Distilled Water	Invitrogen Life Technologies	15230-147
Bovine Serum Albumin (BSA) solution (50 mg/mL)	Invitrogen Life Technologies	15561-020
R-Phycoerythrin Streptavidin	Molecular Probes	S-866
5 M NaCl, RNase-free, DNase-free	Ambion	9760G
PBS, pH 7.2	Invitrogen Life Technologies	20012-027
20X SSPE (3 M NaCl, 0.2 M NaH2PO4, 0.02 M EDTA)	Cambrex	51214
Goat IgG, Reagent Grade	Sigma-Aldrich	I 5256
Anti-streptavidin antibody (goat), biotinylated	Vector Laboratories	BA-0500
Surfact-Amps® 20 (Tween-20), 10%	Pierce Chemical	28320

Supplier Contact Information

Table D.4
Supplier Contact Information

Source	Web Site
ABGene/Marsh	www.marshbio.com
Ambion	www.ambion.com
Affymetrix	www.affymetrix.com
Agencourt	www.agencourt.com
Aldrich	www.sigmaaldrich.com
Ambion	www.ambion.com
Audioadvisor.com	www.audioadvisor.com
Beckman	www.beckman.com
Bio-Rad	www.bio-rad.com
Caliper	www.caliperls.com
Cambrex	www.cambrex.com
Cole-Parmer	www.coleparmer.com
E&K Scientific Products	www.eandkscientific.com
Fisher Scientific	www.fishersci.com
Invitrogen Life Technologies	www.invitrogen.com
Molecular Probes	www.probes.com
Novagen	www.emdbiosciences.com/html/NVG/home.html
Phenix	www.phenix1.com
Pierce Chemical	www.piercenet.com
Promega Corporation	www.promega.com
QIAGEN	www.qiagen.com
Rainin	www.rainin-global.com

Table D.4 (Continued)
Supplier Contact Information

Source	Web Site
Sigma-Aldrich	www.sigma-aldrich.com
USA Scientific	www.usascientific.com
Vector Laboratories	www.vectorlabs.com
VWR International	www.vwr.com

When to Contact Technical Support

Under any of the following conditions, unplug the instrument from the power source and contact Affymetrix Technical Support:

- when the power cord is damaged or frayed;
- if any liquid has penetrated the instrument;
- if, after service or calibration, the instrument does not perform to the specifications stated in *Instrument Specifications* on page 136.

If the instrument must be returned for repair, call Affymetrix Technical Support.

IMPORTANT **Make sure you have the model and serial number.**

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